ELSEVIER

Chemical Geology 231 (2006) 190 – 202

CHEMICAL GEOLOGY INCLUDING ISOTOPE GEOSCIENCE

www.elsevier.com/locate/chemgeo

Carbon and hydrogen isotope variation of plant biomarkers in a plant–soil system

Yoshito Chikaraishi [a,*], Hiroshi Naraoka [b]

[a] *Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology, 2-15 Natsushima-cho, Yokosuka 237-0061, Japan*

[b] *Department of Earth Sciences, Okayama University, 3-1-1 Tsushima-Naka, Okayama 700-8530, Japan*

Received 24 September 2005; received in revised form 7 January 2006; accepted 23 January 2006

Abstract

Stable carbon (δ^{13}C) and hydrogen (δD) isotopic compositions of *n*-alkanes, anteiso-alkanes, *n*-alkanoic acids, *n*-alkanols, phytol and sterols in raw leaves of *Acer argutum* and *Acer carpinifolium*, their fallen leaves, mold and soils from a natural *Acer* forest were measured in order to: (1) understand isotopic variation of the plant biomarkers in a plant–soil system and (2) evaluate which biomarker is the most effective recorder of soil vegetation. Long-chain (>C_{24}) *n*-alkanes, *n*-alkanoic acids and *n*-alkanols are gradually enriched in ^{13}C up to 12.9‰ (average of 4.3‰) and depleted in D up to 94‰ (average of 55‰) from raw leaves to soils. However, anteiso-alkanes, phytol and sterols show little variation in both δ^{13}C (<±1‰) and δD (<±2‰) from raw leaves to soils. These isotope signatures in a plant–soil system indicate that isoprenoid plant biomarkers such as sterols in soils faithfully preserve the isotopic compositions of dominant higher plants growing on the soils without a diagenetic effect upon the isotopic compositions. In contrast, long-chain *n*-alkyl molecules in soils undergo specific isotopic modification during biodegradation associated with early diagenesis and/or a significant contribution from heterotrophic reworking.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Plant–soil system; Carbon isotopic composition; Hydrogen isotopic composition; Plant biomarker; Diagenetic effect

1. Introduction

Stable carbon isotopic compositions (δ^{13}C) of long-chain *n*-alkyl molecules, typically *n*-alkanes such as C_{27}, C_{29}, C_{31} and C_{33}, in soils are widely used as potentially powerful markers for estimating past vegetation changes on the soils (e.g. Lichtfouse, 1997, 1998; Cayet and Lichtfouse, 2001; Wiesenberg et al., 2004; Mead et al., 2005). This is mainly based on the general finding that δ^{13}C values of the *n*-alkyl molecules in soils have a strong correlation with C3/C4 vegetation changes on the soils (e.g. Lichtfouse et al., 1994, 1997; Lichtfouse, 1995). Furthermore, the estimation of vegetation changes provides an assessment for the turnover of organic matter in soil environments (e.g. Lichtfouse, 1997; Wiesenberg et al., 2004). However, there is uncertainty whether plant biomarkers are isotopically preserved during early diagenesis in plant–soil systems. Long exposure of plant debris to oxic conditions before burial accelerates biodegradation of plant molecules in the soil, which may induce isotopic modification of plant biomarkers by heterotrophic degradation and reworking. Generally, unlike low molecular-weight compounds such as phenol and benzoate which show isotopic fractionation during biodegradation (e.g. Hall et al.,

* Corresponding author. Tel.: +81 46 867 9778; fax: +81 867 9775.
E-mail address: ychikaraishi@jamstec.go.jp (Y. Chikaraishi).

0009-2541/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemgeo.2006.01.026

1999; Hunkeler et al., 1999), high molecular-weight compounds such as long-chain *n*-alkanes have been believed to be isotopically stable during biodegradation (Huang et al., 1997; Mazeas et al., 2002). However, some studies report that $\delta^{13}C$ values of long-chain *n*-alkanes in soils were different by several per mil from those of the dominant higher plants growing on the soils in natural environments (Lichtfouse et al., 1995; Ficken et al., 1998; Nguyen Tu et al., 2004). In such cases, long-chain *n*-alkanes in soils are commonly enriched in ^{13}C compared to those of the dominant higher plants. Particularly, Nguyen Tu et al. (2004) recently reported in detail the ^{13}C-enrichment (~3‰) of long-chain *n*-alkanes from raw leaves to fallen leaves and suggested that the $\delta^{13}C$ variation might be strongly affected by diagenesis. Thus, the isotopic stability of plant biomarkers in plant–soil systems has much uncertainty, even though it is essential for reconstruction of past C3/C4 vegetation changes and turnover of organic matter in soils.

Recently, compound-specific hydrogen isotope (δD) analysis (Burgoyne and Hayes, 1998; Hilkert et al., 1999) has been employed as an additional potential tool for studying biomarker sources (Chikaraishi and Naraoka, 2005; Chikaraishi et al., 2005), monitoring biodegradation (Pond et al., 2002) and reconstructing paleoclimatic change (Xie et al., 2000; Andersen et al., 2001; Sauer et al., 2001; Huang et al., 2002, 2004; Sachse et al., 2004; Liu and Huang, 2005; Schefuß et al., 2005). Combined with compound-specific carbon isotope analysis (e.g. Freeman et al., 1990; Hayes et al., 1990; Meier-Augenstein, 1999; Lichtfouse, 2000), δD analysis is an effective tool to help assess the detailed source contribution and fate of organic molecules in natural environments (Chikaraishi and Naraoka, 2005; Chikaraishi et al., 2005). In this study, the carbon and hydrogen isotopic compositions of *n*-alkanes, anteiso-alkanes, *n*-alkanoic acids, *n*-alkanols, phytol and sterols in raw leaves of *Acer argutum* and *Acer carpinifolium*, their fallen leaves, mold and soils from a natural *Acer* forest were measured in order to: (1) understand isotopic variation of the plant biomarkers in a plant–soil system and (2) evaluate which biomarker is the most effective recorder of soil vegetation. The natural forest occupied by the *Acer* species (maples) is chosen as an example of plant–soil system globally distributed in the temperate regions. These molecules are generally abundant in plant leaves (e.g. Chikaraishi et al., 2004a,b), being potential plant biomarkers for estimating past soil environments. The isotopic compositions of *n*-alkanoic acids, *n*-alkanols, phytol and sterols were determined with respect to two preservation forms: solvent-extractable (termed “free”) and saponifiable-released (termed ‘bound’) forms, because the biomarker stability during early diagenesis may depend on the preservation forms (e.g. Eyssen et al., 1973; Cranwell, 1981; Cranwell and Volkman, 1981).

2. Materials and methods

2.1. Study field and samples

Raw leaves of C3 higher plants (*A. argutum* and *A. carpinifolium*), their fallen leaves, mold and soils were collected from a single site in a forest around Lake Haruna located at Gunma Prefecture in Japan (36°28′N, 138°52′E, about 100 km northwest from Tokyo), which was chosen as a location with minimum urban pollution. The two *Acer* trees are the dominant C3 higher plants (deciduous angiosperms) at the field site and no fallen leaves from other species were observed. Raw leaves (fresh green leaves) were collected from the living plants in spring and autumn 1999, in order to determine seasonal isotopic variations. About 20–30 raw leaves were collected from three to five plants each time. About 50 fallen leaves (degraded as light-brown leaves, probably fallen the preceding years) of two *Acer* species were collected from litter in spring 2002. The surface of the raw and fallen leaves was cleaned with distilled water to remove visible contaminants such as dust. Mold (small detrital leaves, probably produced by degradation of leaves from previous years) was collected from the surface layer (0–2 cm in depth) between fallen leaves and soil in spring 2002. Soils were collected from the underlying layer (2–10 cm in depth) in spring and autumn 1999, and in spring 2002, which was brown forest soil with high organic carbon concentrations (>10wt%). Approximately 100 g of mold and 500 g of soil were collected from two to three points each time. All samples were freeze-dried and homogenized to provide sample materials. The sample materials were stored at −20 °C until analysis. $\delta^{13}C$ and δD values of *n*-alkanes, *n*-alkanoic acids, phytol and sterols in the raw leaves for the two *Acer* species were reported in Chikaraishi and Naraoka (2003) and Chikaraishi et al. (2004b).

2.2. Lipid preparation

Lipid preparation followed the procedures of Chikaraishi and Naraoka (2005). Free- and bound-plant biomarkers were differentiated based on operational procedures. In brief, the dried sample was sonicated with CH_2Cl_2/CH_3OH (2/1, v/v, 20 min × 4 times) to

extract free-biomarkers and the residue was subsequently saponified using 0.5 M KOH in CH_3OH/H_2O (95/5, wt/wt) by refluxing (4.5 h) to extract bound-biomarkers. Hydrocarbon (including *n*- and anteiso-alkanes), *n*-alkanoic acid, *n*-alkanol and isoprenoid alcohol fractions (including phytol and sterols) were isolated by silica gel column chromatography, urea adduction and silver nitrate-impregnated silica gel column chromatography. For isotope analysis, the *n*-alkanoic acids and alcohols (i.e. *n*-alkanols, phytol and sterols) were methylesterified with 14% BF_3/CH_3OH or acetylated with acetic anhydride/pyridine (1/1, v/v), respectively (Chikaraishi et al., 2004a,b).

Sterols are not baseline resolved on the complex chromatograms of the isoprenoid alcohol fraction, particularly for soil samples (Fig. 1), which cannot allow for accurate compound-specific isotope determinations. Generally, an independent peak composed of one compound on the chromatogram is needed to determine precise isotopic compositions. Therefore, three sterols: cholest-5-en-3β-ol (cholesterol), 24-methylcholest-5-en-3β-ol (campesterol) and 24-ethylcholest-5-en-3β-ol (β-sitosterol) were further separated from the isoprenoid alcohol fraction by 10 wt.% silver nitrate-impregnated silica gel column chromatography (Chikaraishi and Naraoka, 2005; Chikaraishi et al., 2005).

These plant biomarkers were identified by gas chromatography/mass spectrometry (GC/MS) using an HP 6890 GC connected to an HP MSD 5972A. Their concentrations were quantified using an HP 6890 GC with an on-column injector and a flame-ionization detector (FID) relative to the peak area of external *n*-alkane standards (mixture of 16 *n*-alkanes ranging from C_{18} to C_{36}).

2.3. Compound-specific carbon and hydrogen isotope analyses

Compound-specific carbon and hydrogen isotope analyses were carried out by GC/combustion/isotope ratio mass spectrometry (GC/combustion/IRMS) using a Finnigan Delta S interfaced with HP 5890IIGC and by GC/pyrolysis/IRMS using a Finnigan Delta plus XL interfaced with HP 6890GC, respectively. The combustion was performed in a microvolume ceramic tube with CuO and Pt wires at 840 °C (Hayes et al., 1990). The pyrolysis was performed in a microvolume ceramic tube with graphite at 1440 °C (Burgoyne and Hayes, 1998; Hilkert et al., 1999). $\delta^{13}C$ and δD values are reported in per mil (‰) relative to Peedee Belemnite (PDB) and Standard Mean Ocean Water (SMOW), respectively. Standard deviations of carbon and hydrogen isotope analyses were better than 0.5‰ (~0.3‰ on average) and 7‰ (~3‰ on average), respectively. For *n*-alkanoic

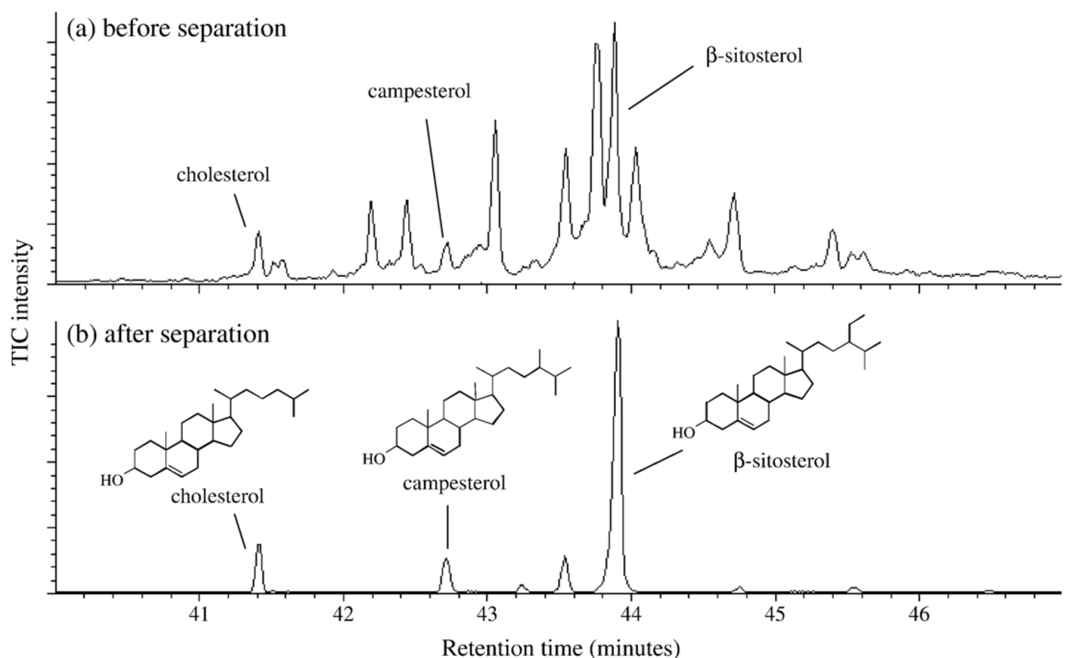

Fig. 1. Representative chromatogram (total ion chromatogram, TIC, on GC/MS analysis) of soil sterols (a) before and (b) after separation by silver nitrate (10 wt.%) impregnated silica gel column chromatography.

acids and alcohols, the contribution of carbon and hydrogen incorporated during derivatization was corrected by an isotopic mass balance calculation, after consideration of the isotopic fractionation associated with the derivatization (Chikaraishi et al., 2004a,b).

3. Results

3.1. Molecular characteristics of plant biomarkers

The concentrations (μg g^{-1} dry sample) of plant biomarkers extracted from the plant–soil samples are summarized in Table 1. Molecular distributions of *n*-alkyl molecules (i.e. *n*-alkanes, *n*-alkanoic acids and *n*-alkanols) are shown in Fig. 2. All samples have a strong predominance of odd carbon-number for *n*-alkanes, and of even carbon-number for *n*-alkanoic acids and *n*-alkanols, which is typically interpreted that these molecules were derived from higher plants. No substantial difference is found in the molecular distribution between the spring and autumn leaves and among the three soil samples. However, the molecular distribution of soils is significantly different from that of raw leaves. For example, the carbon-number with abundance maximum gradually decreases from raw leaves to soils. In particular, a clear shift is observed for *n*-alkane (from C_{31} to C_{29}) and free-*n*-alkanols (from C_{32} to C_{26}). In addition, the carbon preference index (CPI) of *n*-alkanes and *n*-alkanoic acids clearly decreases from raw leaves to soils (Fig. 2). These changes from raw leaves to soils suggest that the biomarker distribution is affected by diagenetic processes such as biodegradation and heterotrophic reworking. C_{30} and C_{32} anteiso-alkanes are found in raw leaves, fallen leaves and mold, but not in soils (Table 1). Although there is a possibility that the anteiso-alkanes are derived from microorganisms, several previous studies reported these anteiso-alkanes originate in higher plant leaves (e.g. Eglinton and Hamilton, 1967; Reddy et al., 2000). In the bound-form biomarkers, *n*-alkanols are detected in fallen leaves, mold and soils, but not in raw leaves. These bound-*n*-alkanols may be derived from free-*n*-alkanols in the plant leaves (for long-chain *n*-alkanols) and/or other soil sources (for short-chain *n*-alkanols) followed by combination into high-

Table 1
Concentrations (μg g^{-1} dry) of lipid biomarkers extracted from the plant–soil samples

Sample	Raw leaves[a]				Fallen leaves[a]		Mold	Soils		
	Aa 99 spring	*Aa* 99 autumn	*Ac* 99 spring	*Ac* 99 autumn	*Aa* 02 spring	*Ac* 02 spring	02 spring	99 spring	99 autumn	02 spring
n-Alkane (free)										
Total of C_{21}–C_{37}	771	239	744	479	220	145	113	5	4	5
Anteiso-alkane (free)										
C_{30}+C_{32}	21	10	42	40	1	4	1	–	–	–
n-Alkanoic acid (free)										
Total of C_{12}–C_{34}	942	577	913	767	741	1028	289	45	73	56
n-Alkanoic acid (bound)										
Total of C_{12}–C_{34}	2945	3489	1789	2806	1490	2194	633	301	351	329
Iso- and anteiso-alkanoic acid (free)										
C_{15}+C_{17}	–	–	–	–	<1	<1	2	2	2	2
Iso- and anteiso-alkanoic acid (bound)										
C_{15}+C_{17}	–	–	–	–	<1	<1	40	31	42	35
n-Alkanol (free)										
Total of C_{18}–C_{34}	382	177	576	295	63	87	38	6	4	5
n-Alkanol (bound)										
Total of C_{18}–C_{34}	–	–	–	–	42	25	66	53	39	46
Isoprenoid (free)										
Phytol	459	381	252	286	<1	<1	<1	–	–	–
Cholesterol	–	–	–	–	<1	<1	<1	<1	<1	<1
Campesterol	57	45	69	40	1	3	1	<1	<1	<1
β-Sitosterol	534	343	221	189	14	16	11	2	2	2
Isoprenoid (bound)										
Phytol	2716	2332	2751	3019	63	53	60	36	40	38
Cholesterol	–	–	–	–	1	<1	1	<1	1	1
Campesterol	20	26	19	18	3	10	6	1	4	3
β-Sitosterol	113	141	70	110	43	44	48	3	28	17

(–) It was not detected.
[a] Full plant names: *Acer argutum* (*Aa*) and *Acer carpinifolium* (*Ac*).

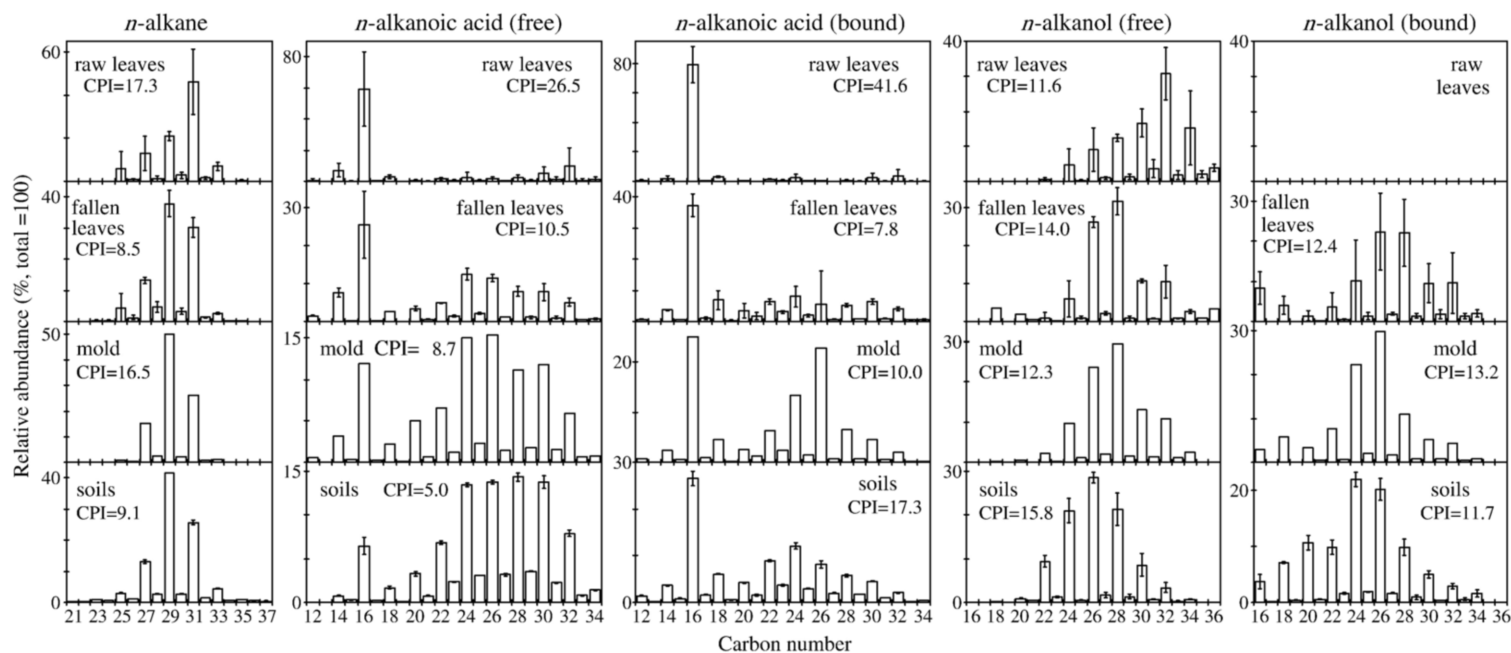

Fig. 2. Histograms of the average molecular distributions of *n*-alkane, *n*-alkanoic acid and *n*-alkanol from plant–soil samples. Histogram and bar represent average and standard deviation of relative abundance (RA) within the same *n*-alkyl group, respectively. CPI = $\sum$RA(odd carbon-numbered homologues)/$\sum$RA(even carbon-numbered homologues) for *n*-alkane or $\sum$RA(even carbon-numbered homologues)/$\sum$RA(odd carbon-numbered homologues) for *n*-alkanoic acid and *n*-alkanol.

Table 2
Carbon isotopic compositions of plant biomarkers extracted from the plant–soil samples (‰, relative to PDB)

Sample	Raw leaves[a]				Fallen leaves[a]		Mold	Soils		
	Aa 99 spring	*Aa* 99 autumn	*Ac* 99 spring	*Ac* 99 autumn	*Aa* 02 spring	*Ac* 02 spring	02 spring	99 spring	99 autumn	02 spring
n-Alkane (free)										
C_{27}	−35.7	−34.9	−35.5	−35.5	−33.6	−33.8	−31.7	−31.5	−32.2	−31.7
C_{29}	−35.9	−35.6	−35.5	−37.3	−34.5	−34.8	−32.6	−32.5	−32.3	−32.5
C_{31}	−36.4	−36.0	−35.4	−37.1	−35.0	−34.6	−33.6	−34.3	−32.8	−33.8
C_{33}	−34.7	−34.0	−35.2	−37.0	−33.2	−34.1	−34.2	−33.0	−32.7	−32.9
Anteiso-alkane (free)										
C_{30}	−32.9	−32.1	−34.4	−34.0	−32.5	−34.0	−33.0			
C_{32}	−32.6	−31.0	−33.8	−33.7	−32.0	−34.0	−32.8			
n-Alkanoic acid (free)										
C_{16}	−35.9	−38.2	−34.5	−39.2	−30.1	−29.5	−29.0	−26.4	−28.3	−27.1
C_{24}	−36.1		−35.9	−37.9	−30.6	−36.8	−32.6	−32.2	−30.8	−31.3
C_{26}	−36.2	−37.6	−36.0	−35.1	−31.9	−36.4	−33.3	−32.3	−32.2	−32.3
C_{28}	−36.8	−35.6	−35.6	−37.5	−32.6	−35.8	−33.8	−34.0	−31.8	−33.8
C_{30}	−36.0	−37.0	−35.7	−38.1	−33.7	−35.9	−35.1	−34.4	−34.0	−34.3
C_{32}		−37.2	−36.1	−37.7	−35.7	−36.5	−35.8	−34.2	−35.1	−34.4
n-Alkanoic acid (bound)										
C_{16}	−36.1	−37.5	−35.0	−39.9	−34.4	−35.3	−32.5	−28.6	−29.1	−28.7
C_{24}	−36.5	−40.1	−36.5	−41.3	−32.4	−36.0	−32.7	−28.7	−28.8	−28.4
C_{26}		−38.4	−37.3	−37.7	−32.1	−34.8	−32.6	−30.5	−30.7	−30.6
C_{28}		−36.3	−35.6	−35.9	−32.7	−35.5	−32.7	−34.3	−31.5	−33.2
C_{30}		−38.0	−35.8	−38.3	−32.8	−36.3	−34.3	−33.2	−34.0	−33.7
C_{32}		−38.1	−36.4	−38.1	−35.8	−37.5	−35.2	−33.0	−33.9	−33.3
n-Alkanol (free)										
C_{26}	−34.1		−35.8	−36.3	−33.8	−34.4	−33.6	−33.7	−32.2	−32.8
C_{28}	−36.0	−35.8	−35.6	−37.0	−35.1	−34.6	−33.9	−33.9	−32.1	−32.3
C_{30}	−35.4	−36.4	−35.3	−38.0	−35.3	−35.0	−33.5	−35.2	−31.6	−33.9
C_{32}	−35.6	−37.1	−35.3	−37.5	−35.8	−35.0	−34.0	−35.2	−33.3	−34.4
n-Alkanol (bound)										
C_{18}					−25.0	−25.4	−26.0	−26.6	−25.0	−25.1
C_{26}					−33.9	−34.3	−33.8	−33.7	−33.6	−33.6
C_{28}					−34.8	−34.4	−33.7	−33.6	−33.2	−33.5
C_{30}					−34.0	−34.2	−32.2	−31.8	−31.8	−31.7
C_{32}					−35.2	−34.6	−34.0	−33.1	−34.5	−33.5
Isoprenoid (free)										
Phytol	−37.9	−37.5	−36.9	−38.8						
Cholesterol								−28.1	−28.7	−28.1
Campesterol	−35.2	−37.3	−33.4	−35.3	−35.4	−34.5	−32.5	−34.9	−36.5	−35.5
β-Sitosterol	−35.6	−35.5	−33.6	−36.0	−35.1	−34.8	−35.3	−35.1	−35.2	−35.2
Isoprenoid (bound)										
Phytol	−40.6	−38.1	−37.8	−37.9	−37.3	−37.7	−37.5	−37.3	−37.2	−37.2
Cholesterol								−25.3	−26.5	−25.3
Campesterol	−34.6	−36.7	−33.6	−35.7	−35.4	−34.8	−35.1	−33.4	−34.9	−34.9
β-Sitosterol	−34.8	−36.3	−34.4	−35.6	−35.5	−34.9	−34.9	−34.7	−34.2	−34.9

[a] Full plant names: *Acer argutum* (*Aa*) and *Acer carpinifolium* (*Ac*).

molecular weight substances such as humic substance during early diagenesis (Lichtfouse et al., 1998a,b,c). Generally, it is known that phytol, campesterol and β-sitosterol are commonly found in higher plant leaves as major isoprenoid constituents, while short-chain iso- and anteiso-alkanoic acids, short-chain *n*-alkanols and cholesterol are found in heterotrophic organisms such as microorganisms and bacteria (e.g. Killops and Killops, 1993, Volkman, 1986, 2003). Therefore, the finding of short-chain iso- and anteiso-alkanoic acids, short-chain *n*-alkanols and cholesterol on fallen leaves, mold and soils in this study suggests heterotrophic activities on the leaf debris and soils. The concentrations of long-chain *n*-alkyl molecules, anteiso-alkanes, phytol, campesterol and β-sitosterol gradually decrease from raw leaves to soils probably due to dilution and biodegradation associated

Table 3
Hydrogen isotopic compositions of plant biomarkers isolated from the plant–soil samples (‰, relative to SMOW)

Sample	Raw leaves[a]				Fallen leaves[a]		Mold	Soils		
	Aa 99 spring	*Aa* 99 autumn	*Ac* 99 spring	*Ac* 99 autumn	*Aa* 02 spring	*Ac* 02 spring	02 spring	99 spring	99 autumn	02 spring
n-Alkane (free)										
C_{27}	−119	−93	−120	−105	−158	−148	−163	−170	−170	−170
C_{29}	−119	−123	−134	−161	−159	−155	−166	−166	−168	−167
C_{31}	−111	−116	−111	−127	−157	−163	−170	−174	−172	−173
C33	−113	−133	−107	−128		−141	−152	−163	−161	−163
Anteiso-alkane (free)										
C_{30}	−172	−188	−171	−189	−183	−180	−181			
C_{32}	−163	−182	−155	−180	−174	−168	−170			
n-Alkanoic acid (free)										
C_{16}	−119	−123	−94	−103	−147	−155	−157	−174	−173	−173
C_{24}	−105		−91	−104	−142	−153	−159	−165	−153	−152
C_{26}			−111	−104	−142	−156	−160	−167	−159	−162
C_{28}	−112		−124	−103	−144	−158	−161	−165	−166	−169
C_{30}	−119	−119	−116	−116	−147	−165	−163	−169	−168	−161
C_{32}		−110	−91	−101	−149	−166	−159	−169	−162	−165
n-Alkanoic acid (bound)										
C_{16}	−107	−125	−114	−127	−148	−148	−150	−159	−159	−159
C_{24}	−101	−127	−96	−121	−132	−142	−158	−159	−163	−167
C_{26}			−99		−144	−163	−160	−176	−167	−176
C_{28}		−75	−118	−101	−151	−164	−168	−166	−168	−165
C_{30}		−114	−117	−125	−151	−156	−171	−170	−171	−172
C_{32}		−108	−81	−97	−137	−164	−160	−175	−169	−174
n-Alkanol (free)										
C_{26}	−148		−117	−135	−160	−150	−167	−177	−167	−179
C_{28}	−115	−106	−123	−126	−135	−145	−156	−170	−159	−167
C_{30}	−117	−106	−138	−139	−137	−154	−160	−165	−175	−165
C_{32}	−119	−108	−119	−115	−115	−117	−117	−167		−185
n-Alkanol (bound)										
C_{18}					−239	−229	−238	−229	−240	−239
C_{26}					−187	−177	−197	−208	−200	−204
C_{28}					−168	−158	−178	−202	−168	−186
C_{30}					−161	−154	−168	−175	−165	−179
C_{32}					−161	−152	−170	−171	−182	−175
Isoprenoid (free)										
Phytol	−323	−343	−333	−337						
Cholesterol								−216	−220	−215
Campesterol	−233	−245	−230	−253	−245	−240	−242	−243	−239	−242
β-Sitosterol	−227	−250	−227	−259	−244	−242	−241	−240	−238	−242
Isoprenoid (bound)										
Phytol	−325	−347	−338	−341	−334	−337	−337	−337	−344	−334
Cholesterol								−218	−214	−216
Campesterol	−238	−251	−234	−239	−239	−241	−242	−244	−243	−240
β-Sitosterol	−236	−255	−229	−236	−247	−239	−248	−244	−242	−239

[a] Full plant names: *Acer argutum* (*Aa*) and *Acer carpinifolium* (*Ac*).

with early diagenesis, where the different relative rates of concentration decrease may depend on distinct stabilities of molecular structures and/or preservation forms. In particular, the absence of anteiso-alkanes and free-phytol in soils suggests that these molecules are quickly degraded during early diagenesis. It is likely that the free-molecules are degraded more quickly than the bound-molecules.

3.2. Carbon and hydrogen isotopic composition of plant biomarkers

Carbon and hydrogen isotopic compositions of the plant biomarkers are summarized in Tables 2 and 3, respectively. The $\delta^{13}C$ values of long-chain (>C_{24}) *n*-alkyl molecules, anteiso-alkanes, phytol, campesterol and β-sitosterol range from −28.4‰ to −41.3‰, which

is consistent with the general $\delta^{13}C$ distribution of lipid molecules from C3 higher plants (e.g. Collister et al., 1994; Lockheart et al., 1997; Ballentine et al., 1998; Chikaraishi and Naraoka, 2003; Conte et al., 2003; Chikaraishi et al., 2004a,b; Bi et al., 2005). Lockheart et al. (1997) reported seasonal $\delta^{13}C$ variations of up to 5.7‰ for long-chain *n*-alkanes within a single plant species, with *n*-alkanes being ^{13}C-depleted for autumn leaves relative to spring leaves and raised a caution for interpretations based on small $\delta^{13}C$ variations of plant

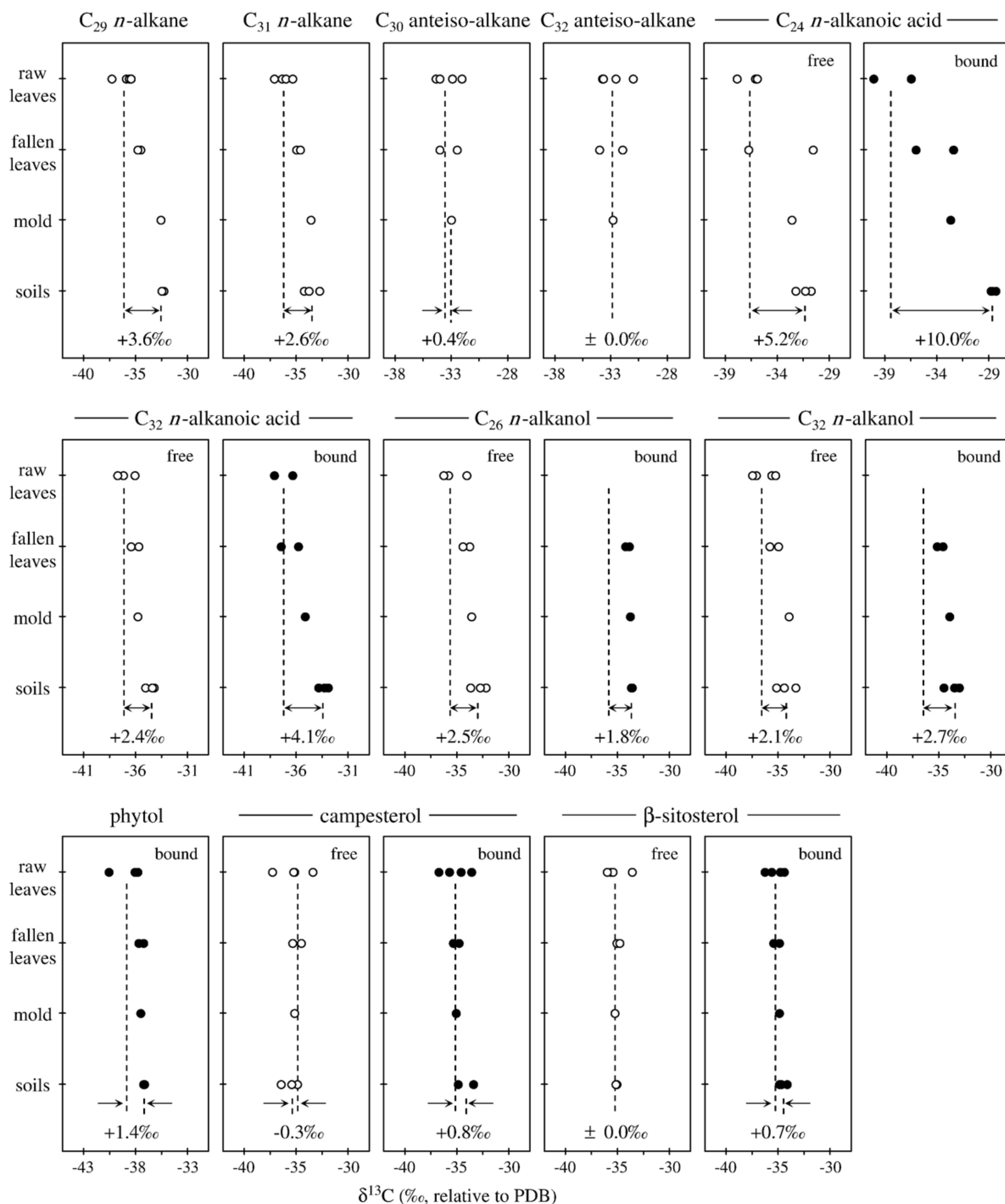

Fig. 3. $\delta^{13}C$ variations of plant biomarkers from raw leaves to soils. The $\delta^{13}C$ shift is calculated by average $\delta^{13}C$ value (as indicated by dash line) between raw leaves and soils (mold for anteiso-alkane). For C_{26} and C_{32} *n*-alkanols, it is calculated with an assumption that $\delta^{13}C$ values of the bound-alkanols for raw leaves are equal to the values of their free-alkanols.

biomarkers. However, in this study, the δ^{13}C and δD variation between spring and autumn leaves for individual molecules is very small (Δ^{13}C autumn–spring = −1.1 ± 1.6‰ and ΔD autumn–spring = −8 ± 13‰) and among three soil samples (Δ^{13}C max–min = 1.1 ± 0.8‰ and ΔD max–min = 8 ± 7‰).

On the other hand, the variation of δ^{13}C and δD values observed from raw leaves to soils is very

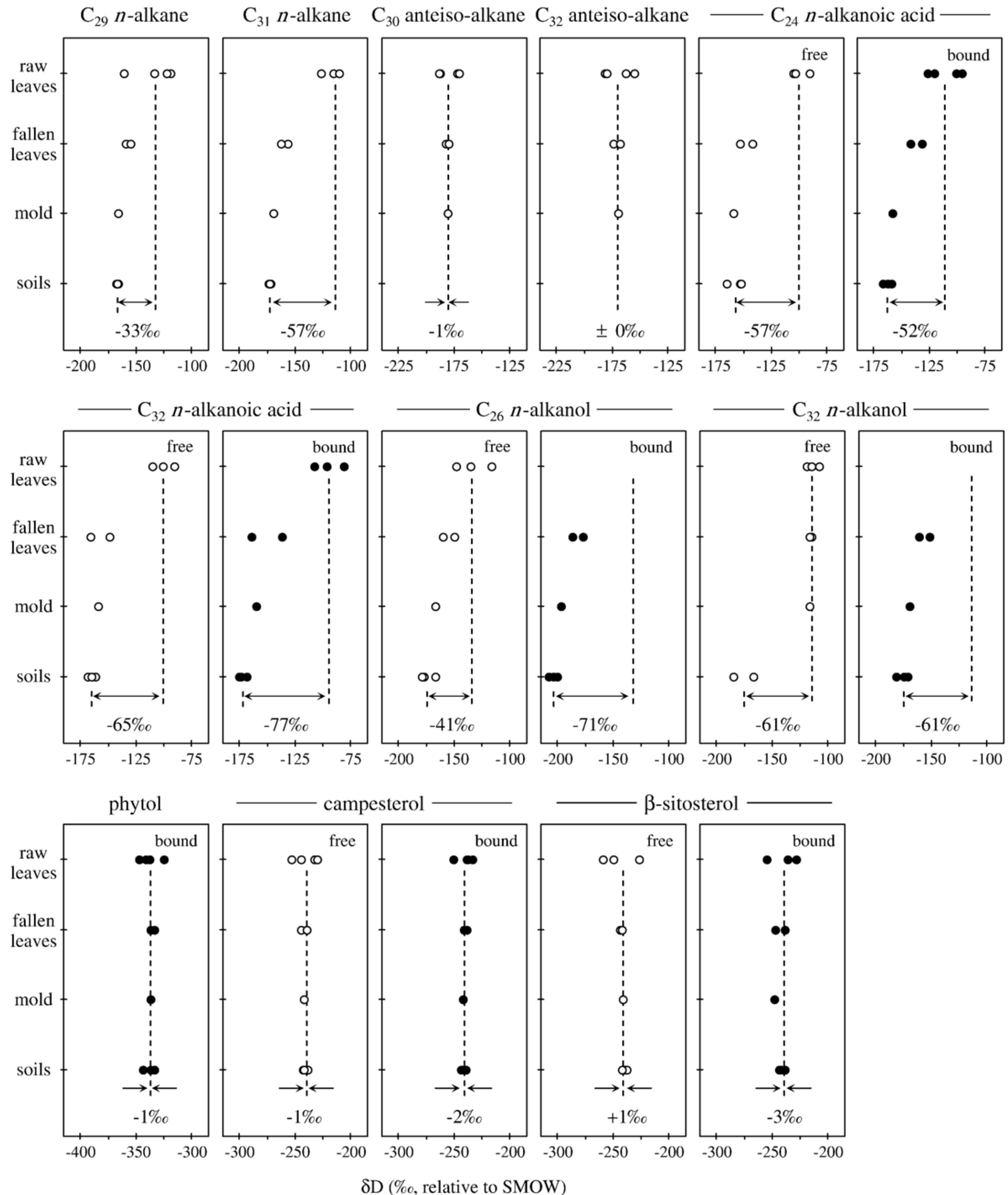

Fig. 4. δD variations of plant biomarkers from raw leaves to soils. The δD shift is calculated by average δD value (as indicated by dash line) between raw leaves and soils (mold for anteiso-alkane). For C_{26} and C_{32} *n*-alkanols, it is calculated with an assumption that δD values of the bound-alkanols for raw leaves are equal to the values of their free-alkanols.

variable. Long-chain *n*-alkyl molecules in soils become enriched in ^{13}C by up to 12.9‰ (average of 4.3‰) and depleted in D by up to 94‰ (average of 55‰) relative to those in raw leaves of *Acer* species growing on the same soils (Figs. 3 and 4). The distinct ^{13}C-enrichment and D-depletion are also observed for the free- and bound-forms of *n*-alkanoic acids and *n*-alkanols with the bound-forms generally showing larger shifts in ^{13}C than the free-forms, but with no systematic shift in δD between the preservation forms. However, the $\delta^{13}C$ and δD values of anteiso-alkanes, phytol, campesterol and β-sitosterol show little variation ($< \pm 1$‰ for $\delta^{13}C$, ±2‰ for δD) from raw leaves to soils (Figs. 3 and 4), i.e. isotope values of soils are essentially identical to the isotope values of the raw leaves.

4. Discussion

4.1. Isotopic modification by diagenesis

For anteiso-alkanes, phytol, campesterol and β-sitosterol, the similar $\delta^{13}C$ and δD compositions in the plant–soil system indicates that these molecules in soils faithfully preserve the isotopic compositions of dominant higher plants growing on the soils without diagenetic effect upon the isotopic compositions. These results are consistent with the conventional wisdom and experimental findings that high molecular-weight compounds are isotopically unaltered during biodegradation (e.g. Huang et al., 1997; Mazeas et al., 2002; Pond et al., 2002). However, ^{13}C-enrichment and D-depletion from raw leaves to soils was observed for three long-chain *n*-alkyl molecules (Tables 1 and 2), in which the extent of the isotopic shifts may depend on the molecular structures and the form of preservation. Although ^{13}C-enrichment for long-chain *n*-alkanes has been reported (Lichtfouse et al., 1995; Ficken et al., 1998; Nguyen Tu et al., 2004), this contradicts the studies with no substantial isotopic change during biodegradation experiments (Huang et al., 1997; Mazeas et al., 2002; Pond et al., 2002).

Besides diagenetic effects such as heterotrophic degradation and reworking, there are three other possibilities that may explain the isotopic shifts of *n*-alkyl molecules: (1) seasonal isotope variation within *Acer* species, (2) isotopic shifts of carbon (CO_2) and hydrogen sources (H_2O) over the past few decades and (3) contribution of C4 plants outside of the plant–soil system. However, the seasonal isotope variation of the same molecules is very small between autumn and spring leaves in this study. Moreover, little isotopic variation of anteiso-alkanes, phytol and sterols in the plant–soil system strongly suggests that isotopic shifts of carbon and hydrogen sources for *Acer* species and possible contribution of C4 vegetation into the plant–soil system are unlikely causes of the isotopic shifts of *n*-alkyl molecules.

Therefore, the diagenetic effects responsible for ^{13}C-enrichment and D-depletion of long-chain *n*-alkyl molecules are specific to these molecules, which may be related to heterotrophic degradation and reworking. Moreover, the presence of short-chain iso- and anteiso-alkanoic acids, short-chain *n*-alkanols and cholesterol (Table 1) in fallen leaf, mold and soil samples probably represents heterotrophic activities on the leaf debris and soils. Generally, high molecular-weight compounds are isotopically unchangeable during biodegradation (e.g. Huang et al., 1997; Mazeas et al., 2002; Pond et al., 2002). However, Sun et al. (2004) recently reported that different biomarkers exhibited distinct patterns of carbon isotopic fractionation during biodegradation experiments, with ^{13}C-enrichment of 2–7‰ for *n*-alkanoic acids, ^{13}C-depletion of 4–6‰ for alkenones and no change for sterols after 40–90% degradation of the molecules. These isotopic changes are similar to the trends of the $\delta^{13}C$ variations of *n*-alkanoic acids and sterols observed in this study, suggesting that long-chain *n*-alkyl molecules in soils may undergo specific isotopic modification during biodegradation associated with early diagenesis. In addition, heterotrophic reworking could also be responsible for the isotopic shifts of *n*-alkyl molecules in the plant–soil system. A variety of heterotrophic soil organisms such as insects, bacteria and fungi produce various long-chain *n*-alkyl molecules. For example, insects such as termites produce C_{25}, C_{27} and C_{29} *n*-alkanes (e.g. Jurenka and Subchev, 2000; Subchev and Jurenka, 2001; Szafranek et al., 2001; Kaib et al., 2002), and C_{32} *n*-alkanols (e.g. Szafranek et al., 2001). Several soil heterotrophic bacteria and fungi produce C_{22}, C_{24} and C_{26} *n*-alkanoic acids (e.g. Rezanka et al., 1991; Rezanka and Sokolov, 1993; Muchembled et al., 2000). It is likely that the relative contribution of long-chain *n*-alkyl molecules from heterotrophic reworking becomes increasingly significant with progressive decay of plant leaves, which may also explain the changes in relative molecular distributions (e.g. carbon number with abundance maximum and CPI values) of this study (Fig. 2). A dramatic increase of the abundance ratio of *n*-alkanes ($C_{29}+C_{31}$) vs. anteiso-alkanes ($C_{30}+C_{32}$) (Fig. 5) indicates additional inputs of *n*-alkanes into this plant–soil system besides the dominant plant leaves, because *n*-alkanes should be degraded faster than anteiso-alkanes (e.g. Schaeffer et al., 1979; Aggarwal and Hinchee, 1991). Moreover, C_{18} *n*-alkanol (probably derived from soil heterotrophs) has $\delta^{13}C$ values of

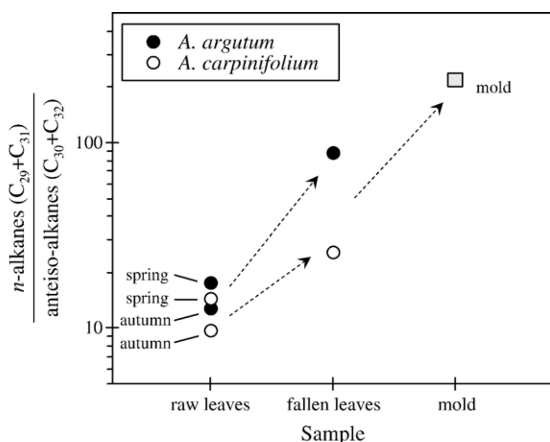

Fig. 5. Abundance ratio of n-alkanes ($C_{29}+C_{31}$) vs. anteiso-alkanes ($C_{30}+C_{32}$) from raw leaves to mold.

~−25.5‰ and δD values of ~−236‰ in fallen leaf, mold and soil samples (Tables 2 and 3), being enriched in ^{13}C and depleted in D relative to long-chain n-alkyl molecules of *Acer* leaves. When long-chain n-alkyl molecules from the heterotrophic reworking are also ^{13}C-enriched and D-depleted relative to those from *Acer* leaves, the ^{13}C-enrichment and D-depletion from raw leaves to soils can be explained by a mixing of both contribution.

4.2. Implications for the soil vegetation record

Diagenetic effects on the isotopic compositions of plant biomarkers in plant–soil systems have are poorly understood, even though significant degradation of the biomarkers might occur during exposure to oxic conditions. For example, considerable isotopic changes of n-alkyl molecules during migration from plant to soil are observed in this study (Figs. 4 and 5) as well as previous studies (e.g. Lichtfouse et al., 1995; Ficken et al., 1998; Nguyen Tu et al., 2004), suggesting a common isotope gradient for n-alkyl molecules in natural plant–soil systems. These results indicate the necessity for circumspection during reconstruction of possible past C3/C4 vegetation changes and the turnover of organic matter in soils based on δ^{13}C changes of n-alkyl molecules. Detailed biodegradation and reworking experiments are needed to clarify the isotopic modification of n-alkyl molecules in plant–soil systems. In contrast, anteiso-alkanes, phytol, campesterol and β-sitosterol in soils faithfully preserve the isotopic compositions of dominant higher plants growing on the soils without diagenetic effect upon the isotopic compositions (Figs. 4 and 5). An important requirement for environmental indicators of past conditions is that biomarkers should be isotopically unchanged during diagenesis. Therefore, anteiso-alkanes, phytol, campesterol and β-sitosterol will be more suitable plant biomarkers to reconstruct past C3/C4 vegetation changes and turnover of organic matter in soils. In particular, since bound-sterols show greater resistance to biodegradation in the soils compared to anteiso-alkanes and phytol (Table 1), bound-sterols such as campesterol and β-sitosterol will be the most effective proxies of soil vegetation.

5. Conclusions

Dual isotope (δ^{13}C–δD) analysis of various plant biomarkers provides more detailed information on their isotopic variations in plant–soil systems. Two important results of this study are summarized as follows:

(1) Similar to previous studies (Lichtfouse et al., 1995; Ficken et al., 1998; Nguyen Tu et al., 2004), long-chain (>C_{24}) n-alkanes, n-alkanoic acids and n-alkanols are gradually enriched in ^{13}C (average of 4.3‰) and depleted in D (average of 55‰) from raw leaves to soils, suggesting a common isotope gradient for n-alkyl molecules in natural plant–soil systems. Although detailed biodegradation and reworking experiments are needed to clarify this isotopic modification in plant–soil systems, this study clearly suggests the necessity for circumspection during reconstruction of past C3/C4 vegetation changes and turnover of organic matter in soils based on δ^{13}C values of n-alkyl molecules.

(2) Compared to the long-chain n-alkyl molecules, both δ^{13}C and δD values of anteiso-alkanes, phytol, campesterol and β-sitosterol show little variation from raw leaves to soils, which indicates that these molecules in soils faithfully preserve the isotopic compositions of dominant higher plants growing on the soils without diagenetic effect upon the isotopic compositions. These results suggest that isoprenoid plant biomarkers such as campesterol and β-sitosterol will be more suitable plant biomarkers to reconstruct past C3/C4 vegetation changes and turnover of organic matter in soils.

Acknowledgment

We thank Mr. Naito for the assistance in the field. We are grateful to Prof. S.R. Poulson for constructive comments and English improvement, and to Dr. N. Ohkouchi, Dr. N.O. Ogawa, Dr. K. Matsumoto and Dr. H. Kitazato for expert advice and constructive

discussion. We are also grateful to two anonymous reviewers for their valuable suggestions and critical comments. This work was supported by a research fellowship of the Japan Society for the Promotion of Science for Young Scientists (Y.C.) and the Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Culture and Technology (H.N.). **[PD]**

References

Andersen, N., Paul, H.A., Bernasconi, S.M., Mackenzie, J.A., Behrens, A., Schaeffer, P., Albrecht, P., 2001. Large and rapid climate variability during the Messinian salinity crisis: evidence from deuterium concentrations of individual biomarkers. Geology 29, 799–802.

Aggarwal, P., Hinchee, R.E., 1991. Monitoring in situ biodegradation of hydrocarbons by using stable carbon isotopes. Environ. Sci. Technol. 25, 1178–1180.

Ballentine, D.C., Macko, S.A., Turekian, V.C., 1998. Variability of stable carbon isotopic compositions in individual fatty acids from combustion of C4 and C3 plants: implications for biomass burning. Chem. Geol. 152, 151–161.

Bi, X., Sheng, G., Liu, X., Li, C., Fu, J., 2005. Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. Org. Geochem. 36, 1405–1417.

Burgoyne, T.W., Hayes, J.M., 1998. Quantitative production of H_2 by pyrolysis of gas chromatographic effluents. Anal. Chem. 70, 5136–5141.

Cayet, C., Lichtfouse, É., 2001. δ^{13}C of plant-derived *n*-alkanes in soil particle-size fractions. Org. Geochem. 32, 253–258.

Chikaraishi, Y., Naraoka, H., 2003. Compound-specific δD–δ^{13}C analyses of *n*-alkanes extracted from terrestrial and aquatic plants. Phytochemistry 63, 361–371.

Chikaraishi, Y., Naraoka, H., 2005. δ^{13}C and δD identification of sources of lipid biomarkers in sediments of Lake Haruna (Japan). Geochim. Cosmochim. Acta 69, 3285–3297.

Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004a. Carbon and hydrogen isotopic fractionation during lipid biosynthesis in a higher plant (*Cryptomeria japonica*). Phytochemistry 65, 323–330.

Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004b. Hydrogen and carbon isotopic fractionations of lipid biosyntheses among terrestrial (C3, C4 and CAM) and aquatic plants. Phytochemistry 65, 1369–1381.

Chikaraishi, Y., Yamada, Y., Naraoka, H., 2005. Carbon and hydrogen isotopic compositions of sterols from riverine and marine sediments. Limnol. Oceanogr. 50, 1763–1770.

Collister, J.W., Rieley, G., Stern, B., Eglinton, G., Fry, B., 1994. Compound-specific δ^{13}C analyses of leaf lipids from plants with differing carbon dioxide metabolisms. Org. Geochem. 21, 619–627.

Conte, M.H., Weber, J.C., Carlson, P.J., Flanagan, L.B., 2003. Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem. Oecologia 135, 67–77.

Cranwell, P.A., 1981. Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. Org. Geochem. 3, 79–89.

Cranwell, P.A., Volkman, J.K., 1981. Alkyl and steryl esters in a recent lacustrine sediment. Chem. Geol. 32, 29–43.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322–1335.

Eyssen, H.J., Parmentier, G.G., Compernolle, F.C., 1973. Biohydrogenation of sterols by *Eubacterium* ATCC 21,408-*Nova* species. Eur. J. Biochem. 36, 411–421.

Ficken, K.J., Barber, K.E., Eglinton, G., 1998. Lipid biomarker, δ^{13}C and plant macrofossil stratigraphy of a Scottish montane peat bog over the last two millennia. Org. Geochem. 28, 217–237.

Freeman, K.H., Hayes, J.M., Trendel, J.-M., Albrecht, P., 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. Nature 343, 254–256.

Hall, J.A., Kalin, R.M., Larkin, M.J., Allen, C.C.R., Harper, D.B., 1999. Variation in stable carbon isotope fractionation during aerobic degradation of phenol and benzoate by contaminant degrading bacteria. Org. Geochem. 30, 801–811.

Hayes, J.M., Freeman, K.H., Popp, B.N., Hoham, C.H., 1990. Compound-specific isotopic analyses: a novel tool for reconstruction of ancient biogeochemical processes. Org. Geochem. 16, 1115–1128.

Hilkert, A.W., Douthitt, C.B., Schlüter, H.J., Brand, W.A., 1999. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 13, 1226–1230.

Huang, Y., Eglinton, G., Ineson, P., Latter, P.M., Bol, R., Harkness, D.D., 1997. Absence of carbon isotope fractionation of individual *n*-alkanes in a 23-year field decomposition experiment with *Calluna vulgaris*. Org. Geochem. 26, 497–501.

Huang, Y., Shuman, B., Wang, Y., Webb III, T., 2002. Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late Quaternary climate variations. Geology 30, 1103–1106.

Huang, Y., Shuman, B., Wang, Yi., Webb III, T., 2004. Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test. J. Paleolimnol. 31, 363–375.

Hunkeler, D., Aravena, R., Butler, B.J., 1999. Monitoring microbial dechlorination of tetrachloroethene (PCE) in groundwater using compound-specific stable carbon isotope ratios: microcosm and field studies. Environ. Sci. Technol. 33, 2733–2738.

Jurenka, R.A., Subchev, M., 2000. Identification of cuticular hydrocarbons and the alkene precursor to the pheromone in hemolymph of the female gypsy moth, *Lymantria dispar*. Arch. Insect Biochem. Physiol. 43, 108–115.

Kaib, M., Franke, S., Franke, W., Brandl, R., 2002. Cuticular hydrocarbons in a termite: phenotypes and a neighbour-stranger effect. Physiol. Entomol. 27, 189–198.

Killops, S.D., Killops, V.J., 1993. Chemical composition of biogenic matter. In: Gill, R.C.O. (Ed.), An Introduction to Organic Geochemistry. John Wiley & Sons, Inc., New York, pp. 22–62.

Lichtfouse, É., 1995. ^{13}C labeling of soil *n*-hentriacontane (C_{31}) by maize cultivation. Tetrahedron Lett. 36, 529–530.

Lichtfouse, É., 1997. Heterogeneous turnover of molecular organic substances from crop soils as revealed by ^{13}C labeling at natural abundance with *Zea mays*. Naturwissenschaften 84, 23–35.

Lichtfouse, É., 1998. Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soils. Naturwissenschaften 85, 76–77.

Lichtfouse, É., 2000. Compound-specific isotope analysis. Application to archaeology, biomedical science, biosynthesis, environment, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. Rapid Commun. Mass Spectrom. 14, 1337–1344.

Lichtfouse, É., Elbisser, B., Balesdent, J., Mariotti, A., Bardoux, G., 1994. Isotope and molecular evidence for direct input of maize leaf wax *n*-alkanes into crop soils. Org. Geochem. 22, 349–351.

Lichtfouse, É., Dou, S., Girardin, C., Grably, M., Balesdent, J., Behar, F., Vandenbroucke, M., 1995. Unexpected ^{13}C-enrichment of organic components from wheat crop soils: evidence for the in situ origin of soil organic matter. Org. Geochem. 23, 865–868.

Lichtfouse, É., Bardoux, G., Mariotti, A., Balesdent, J., Ballentine, D.C., Macko, S.A., 1997. Molecular, ^{13}C and ^{14}C evidence for the allochthonous and ancient origin of C_{16}–C_{18} *n*-alkanes in modern soil. Geochim. Cosmochim. Acta 61, 1891–1898.

Lichtfouse, É., Chenu, C., Baudin, F., Leblond, C., Silva, M.A., Behar, F., Derenne, S., Largeau, C., Wehrung, P., Albrecht, P., 1998a. A novel pathway of soil organic matter formation by selective preservation of resistant straight-chain biopolymers: chemical and isotope evidence. Org. Geochem. 28, 415–441.

Lichtfouse, É., Wehrung, P., Albrecht, P., 1998b. Plant wax *n*-alkanes trapped in soil humin by noncovalent bonds. Naturwissenschaften 85, 449–452.

Lichtfouse, É., Leblond, C., Silva, M.D., Behar, F., 1998c. Occurrence of biomarkers and straight-chain biopolymers in Humin: implication for the origin of soil organic matter. Naturwissenschaften 85, 497–501.

Liu, W., Huang, Y., 2005. Compound-specific D/H ratios and molecular distributions of higher plant leaf waxes as novel paleoenvironmental indicators in the Chinese Loess Plateau. Org. Geochem. 36, 851–860.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P., 1997. Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: implications for the study of higher land plant-derived sedimentary organic matter. Org. Geochem. 26, 137–153.

Mazeas, L., Budzinski, H., Raymond, N., 2002. Absence of stable carbon isotope fractionation of saturated and polycyclic aromatic hydrocarbons during aerobic bacterial biodegradation. Org. Geochem. 33, 1259–1272.

Mead, R., Xu, Y., Chong, J., Jaffé, R., 2005. Sediment and soil organic matter source assessment as revealed by the molecular distribution and carbon isotope composition of *n*-alkanes. Org. Geochem. 36, 363–370.

Meier-Augenstein, W., 1999. Applied gas chromatography coupled to isotope ratio mass spectrometry. J. Chromatogr., A 842, 351–371.

Muchembled, J., Sahraoui, L.-H., Grandmougin-Ferjani, A., Sancholle, M., 2000. Effect of age on the fatty acids content of *Blumeria graminis* conidia. Biochem. Soc. Trans. 28, 875–877.

Nguyen Tu, T.T., Derenne, S., Largeau, C., Bardoux, G., Mariotti, A., 2004. Diagenesis effects on specific carbon isotope composition of plant *n*-alkanes. Org. Geochem. 35, 317–329.

Pond, K., Huang, Y., Wang, Y., Kulpa, C.F., 2002. Hydrogen isotope composition of individual *n*-alkanes as an intrinsic tracer for bioremediation and source identification of petroleum contamination. Environ. Sci. Technol. 36, 724–728.

Reddy, C.M., Eglinton, T.I., Palic, R., Benitez-Nelson, B.C., Stojanovic, G., Palic, I., Djordjevic, S., Eglinton, G., 2000. Even carbon number predominance of plant wax *n*-alkanes: a correction. Org. Geochem. 31, 331–336.

Rezanka, T., Sokolov, M.Y., 1993. Rapid method for the enrichment of very long-chain fatty acids from microorganisms. J. Chromatogr., A 636, 249–254.

Rezanka, T., Zlatkin, I.V., Víden, I., Slabov, O.I., Nikitin, D.I., 1991. Capillary gas chromatography–mass spectrometry of unusual and very long-chain fatty acids from soil oligotrophic bacteria. J. Chromatogr., A 558, 215–221.

Sachse, D., Radke, J., Gleixner, G., 2004. Hydrogen isotope ratios of recent lacustrine sedimentary *n*-alkanes record modern climate variability. Geochim. Cosmochim. Acta 68, 4877–4889.

Sauer, P., Eglinton, T.I., Hayes, J.M., Schimmelmann, A., Sessions, A.L., 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental climatic conditions. Geochim. Cosmochim. Acta 65, 213–222.

Schaeffer, T.L., Cantwell, S.G., Brown, J.F., Watt, D.S., Fall, R.R., 1979. Microbial growth on hydrocarbons: terminal branching inhibits biodegradation. Appl. Environ. Microbiol. 38, 742–746.

Schefuß, E., Schouten, S., Schneider, R.R., 2005. Climatic controls on central African hydrology during the past 20,000 years. Nature 427, 1003–1006.

Subchev, M., Jurenka, R.A., 2001. Sex pheromone levels in pheromone glands and identification of the pheromone and hydrocarbons in the hemolymph of the moth *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 47, 35–43.

Sun, M.-Y., Zou, L., Dai, J., Ding, H., Culp, R.A., Scranton, M.I., 2004. Molecular carbon isotopic fractionation of algal lipids during decomposition in natural oxic and anoxic seawaters. Org. Geochem. 35, 895–908.

Szafranek, B., Maliñski, E., Nawrot, J., Sosnowska, D., Ruszkowska, M., Pihlaja, K., Trumpakaj, Z., Szafranek, J., 2001. In vitro effect of cuticular lipids of the aphids *Sitobion avenae*, *Hyalopterus pruni* and *Brevicoryne brassicae* on growth and sporulation of the *Paecilomyces fumosoroseus* and *Beauveria bassiana*. ARKIVOC 3, 81–94.

Volkman, J.K., 1986. A review of sterol markers for marine and terrigenous organic matter. Org. Geochem. 9, 83–99.

Volkman, J.K., 2003. Sterols in microorganisms. Appl. Microbiol. Biotechnol. 60, 495–506.

Wiesenberg, G.L.B., Schwarzbauer, J., Schmidt, M.W.I., Schwark, L., 2004. Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. Org. Geochem. 35, 1371–1393.

Xie, S., Nott, C.J., Avsejs, L.A., Volders, F., Maddy, D., Chambers, F.M., Gledhill, A., Carter, J.F., Evershed, R.P., 2000. Palaeoclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. Org. Geochem. 31, 1053–1057.