Mass spectrometric characterization of $^{13}$C-labeled lipid tracers and their degradation products in microcosm sediments

M.-Y. Sun *

Department of Marine Sciences, The University of Georgia, Athens, GA 30602, USA

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Abstract

A GC–MS approach was developed to characterize $^{13}$C-labeled tracers in microcosm sediments. Three major $^{13}$C-labeled fatty acids (16:1o7, 16:0, and 18:1o9) and phytol in labeled microalgae (added to sediments as organic substrate) were distinguished from natural sedimentary counterparts. During a series of incubations under various redox conditions (oxic and anoxic) and with and without the presence of macrofauna (i.e. Yoldia limiatula), two newly produced $^{13}$C-labeled compounds (iso-15:0 fatty acid and C16 alcohol) were identified from incubated sediments. Analyses of mass spectra showed that the iso-15:0 fatty acid was partially labeled while the C16 alcohol was uniformly labeled with $^{13}$C, implying that the formation pathways of these two degradation products may be different. Estimates of degradation rate constants of planktonic lipids during these incubations showed that oxic conditions and the presence of Yoldia significantly accelerated the degradation of planktonic lipids in sediments. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: $^{13}$C-labeled microalgae; Lipids; Degradation products; Rate constants; Redox conditions; Benthic macrofauna

1. Introduction

The decomposition of organic matter is a driving force for diagenetic processes in marine sediments (Berner, 1980). The enriched tracer approach has been used widely to determine rates and pathways of organic matter degradation. The use of radiotracers such as labeled algal material as well as specific compounds has been successful (Rhead et al., 1971; Brooks and Maxwell, 1974; Gaskell and Eglinton, 1975; Edmunds et al., 1980, Taylor et al., 1981; Henrichs and Doyle, 1986; Harvey et al., 1986; Sun et al., 1993, 1997; Kristensen et al., 1995). However, the main drawback of the radiotracer technique involves the difficulties of using radioactive compounds in the same instruments used for structural elucidation.

Recent advances in the synthesis of stable isotopically labeled compounds or algal material have promoted the application of enriched stable isotopic tracers in geochemical studies. For example, Blair et al. (1996) used uniformly labeled microalgae ($^{13}$C > 98%) as a labile carbon tracer to track the short-term fate of fresh organic matter in continental slope sediments. They primarily followed the changes of $^{13}$C signature in POC and $\Sigma$CO$_2$. However, the potential for using the $^{13}$C-tracer technique to study the degradation and transformation of organic matter at the molecular level would be enhanced if gas chromatography–mass spectrometry (GC–MS) or gas chromatography/isotope ratio mass spectrometry (GS–IRMS) were used.

Until now, few studies have been conducted on the development of GC–MS characterization of $^{13}$C-labeled tracers and their degradation products from natural or experimental systems. This study is a first attempt to establish an effective GC–MS procedure to characterize

* Corresponding author Tel.: +1-706-542-5709; fax: +1-706-542-5888.
E-mail address: mysun@arches.uga.edu
$^{13}$C-labeled lipid compounds contained in labeled microalgal material and to distinguish them from their natural counterparts. This GC–MS technique was used to identify several newly produced compounds during a series of microcosm experiments, to help us understand the decomposition mechanisms of organic matter in sediments. In addition, this method was applied to estimate degradation rate constants of microalgal lipids in sediments under various biochemical conditions, illustrating its great application in organic geochemistry.

2. Experimental

2.1. Materials and incubation samples

Uniformly labeled microalgae (Chlorella $^{13}$C > 98%) was obtained from Cambridge Isotope Laboratories. Specimens of Yoldia limatula (1–1.4 cm in size used in experiments) were obtained from the Marine Biology Laboratory (Woods Hole, MA). The surface (top 1–2 cm) sediments were collected from central Long Island Sound (LIS). The organic carbon content of this surface sediment is approximately 2% (Sun et al., 1994). Labeled microalgae was mixed with wet sediment (1 g:200 g by weight), which increased the organic content of sediments by $\sim$0.5%. The mixed sediments were made into several thin ($\sim$5 mm) cakes. These cakes were frozen overnight and then introduced on top of incubation microcosms. About 20 mg of labeled microalgae and $\sim$5 g natural bulk sediments (not mixed with labeled tracer) were extracted separately for use in analyses of $^{13}$C-labeled and natural unlabeled lipids.

Details of the microcosm set-up are described elsewhere (Sun et al., 1999). In brief, the microcosm consisted of several sets of subcores with labeled microalgae added at the sediment–water interface as a thin sediment cake (0.5 cm thick). Two sets of incubation experiments were conducted during October 1996 and March 1997. In the October 1996 incubation, the abundance of Yoldia in a series of subcores was 690/m$^2$, which corresponds with its natural abundance in LIS sediments. In the March 1997 incubation, the abundance of Yoldia in three series of subcores varied in the range of 200–1200/m$^2$. Control subcores in these incubations had no Yoldia added. Subcores were removed from an incubation chamber over a time series (0, 5, 10, 15 and 29 days) whereby the sediment cores were sectioned into several intervals. Suspended particles, which were ejected by Yoldia (Bender and Davis, 1984), were collected from the overlying water.

In another set of incubations (August 1997), the mixed sediments were made into thin PVC holders (1.5 mm thick, 8 cm i.d.), which were incubated in water tanks under variable redox conditions (oxic and anoxic). The control for redox conditions in this incubation was described in previous papers (Aller and Mackin, 1989; Sun et al., 1993). Incubated sediment samples and suspended particles were stored frozen (–20°C) for later extraction.

2.2. Lipid analysis

The incubated samples (~1–2 g) were thawed and extracted with 10 ml methanol, followed by 3 × 10 ml methylene chloride-methanol (2:1 v/v). The combined extracts were partitioned into a methylene chloride phase by addition of 5% NaCl solution. After the methylene chloride phase was dried, lipids were saponified with KOH/methanol at 100°C for 2 h to release fatty acids and fatty alcohols from various esters and other matrices. Neutral compounds (i.e. hydrocarbons, alcohols, sterols, etc.) were extracted from the basic solution (pH > 13) while fatty acids were extracted following the addition of HCl (pH < 2). Fatty acid extracts were methylated with BF$_3$–MeOH to form fatty acid methyl esters (FAMEs). Neutral extracts were further separated into several fractions through SiO$_2$ column chromatography. Alcohols were silylated with BSTFA in acetonitrile to form TMS-ethers.

FAMEs and alcohol-TMS-ethers were first analyzed by capillary gas chromatography using a Hewlett-Packard 6890 gas chromatograph with an on-column injector and flame ionization detector. Separations were achieved with a 30 m × 0.25 mm i.d. column coated with 5% phenyl methyl silicone (HP-5, Hewlett-Packard) with H$_2$ as carrier gas. The temperature was programmed to 50–150°C at 20°C/min followed by 150–320°C at 4°C/min and a 5 min hold at 310°C. Internal standards [5a(H)-cholestan for alcohol-TMS-ethers and nonadecanoic acid methyl ester for FAMEs] were added to samples immediately prior to GC analysis to aid in quantification. GC–MS analysis was performed on a Hewlett-Packard 5890/Finnigan Incos 50 GC–MS system with a 30 m × 0.25 mm i.d. DB-5 column and He as carrier gas. Operating conditions were: mass range 50–650 with a 1 s cycle time; 70 eV ionizing energy to result in electron ionization (EI); GC temperature program for FAMEs (80–180°C at 20°C/min, 180–270°C at 4°C/min, 270–310°C at 10°C/min and a 5 min hold at 310°C) and for alcohol-TMS-ethers (80–150°C at 20°C/min followed by 150–320°C at 4°C/min and a 5 min hold at 320°C).

3. Results and discussion

3.1. Characterization of $^{13}$C-labeled algal lipids

The key to distinguishing $^{13}$C-enriched and natural counterparts is the difference in $m/z$ of their characteristic
Fig. 1 shows the mass spectra of natural unlabeled, uniformly-\textsuperscript{13}C-labeled, and mixed (ratio of \textsuperscript{13}C:\textsuperscript{12}C = 8:1) 16:0 fatty acid methyl ester. Saturated FAMEs in natural sediments give a base peak at \( m/z = 74 \) \([\text{CH}_2=\text{C(OH)}-\text{O-CH}_3]^+\), which is formed through a rearrangement of a radical ion (McLafferty, 1963). The formation mechanism involves a specific transfer of a \( \gamma \)-hydrogen atom in a six-membered transition state to the carbonyl oxygen atom with subsequent cleavage at the \( \beta \)-carbon atom. The \( m/z \) of the corresponding fragment for the uniformly labeled FAME derived from labeled algae is at \( m/z = 76 \) \([\text{13CH}_2=\text{13C(OH)}-\text{O-CH}_3]^+\). Two carbon atoms from a fatty acid chain are labeled with \textsuperscript{13}C while the carbon in the methoxy group of this fragment originates from unlabeled methanol used for methylation. Another pair of fragments with high
intensity for natural and labeled 16:0 FAME were at m/z = 87 and 90, representing [CH$_2$-CH$_2$(CO)-O-CH$_3$]$^+$ and [\(^{13}\)CH$_2$-\(^{13}\)CH$_2$-(\(^{13}\)CO)-O-CH$_3$]$^+$ respectively. They are formed through simple cleavage of the FAME chain (McLafferty, 1963).

When labeled tracers were mixed with natural sediments, the fragment pairs ($m/z = 74$ and 76; $m/z = 87$ and 90) occurred simultaneously in mass spectra (Fig. 1). Thus, the differences in $m/z$ (2-unit and 3-unit) of these fragments between \(^{13}\)C-FAMEs and unlabeled FAMEs serves as the basis for distinguishing \(^{13}\)C-labeled and natural saturated FAMEs. At the operational ionizing energy (70 eV), the amount of molecular ions produced during electron ionization is relatively small, so they were not used. For unlabeled and \(^{13}\)C-labeled 16:1 and 18:1 FAMEs, the base peaks were at $m/z = 55$ and 59 ([$CH_2$-CH = CH-CH$_3$]$^+$ and [\(^{13}\)CH$_2$-\(^{13}\)CH = \(^{13}\)CH-\(^{13}\)CH$_3$]$^+$) respectively, but the fragments with $m/z = 74$ and 76 were still abundant. Thus, the differences in $m/z$ (4-unit and 2-unit) of these two pairs of fragments were used to distinguish \(^{13}\)C-labeled and natural unsaturated FAMEs.

Fig. 2 shows the mass spectra of natural unlabeled, uniformly \(^{13}\)C-labeled, and mixed (ratio of \(^{13}\)C:12C = 1:7:1) phytol-TMS-ether. The spectrum of natural unlabeled phytol-TMS-ether is consistent with that reported in the literature (McLafferty and Stauffer, 1989) and is confirmed to have trans-geometry using an authentic standard. The base peak of unlabeled phytol-TMS-ether occurs at $m/z = 143$, which represents a silylated unit [CH$_2$-CH = CH-CH$_2$O-Si-(CH$_3$)$_3$]$^+$ (Fig. 2). The base peak of the corresponding fragment from \(^{13}\)C-labeled phytol-TMS-ether shifts to $m/z = 147$, because four carbon atoms originate from the \(^{13}\)C-phytol skeleton while three methyl groups come from the unlabeled BSTFA reagent during silylation. When \(^{13}\)C-labeled microalgal extract was mixed with natural sediment extract, the fragments with $m/z = 143$ and 147 occurred simultaneously in the mass spectra.

3.2. Calibration for distinguishing \(^{13}\)C-labeled from natural lipids in experimental sediments

The ratios between the relative intensities of these characteristic fragments (e.g., $m/z = 76:74$ and 90:87 for saturated FAMEs; $m/z = 59:55$ and 76:74 for unsaturated FAMEs; and $m/z = 147:143$ for phytol-TMS-ether) reflect the relative proportion of \(^{13}\)C-labeled to unlabeled compounds. In this study, mass spectra of target compounds were obtained by scanning the entire area rather than on the peak maximum. When \(^{13}\)C-tracers were mixed with natural sediments, the compound peaks became slightly wider compared to those of natural compounds alone. However, no split peaks were observed although the ratio of \(^{13}\)C to $^{12}$C might vary dramatically.

In order to distinguish \(^{13}\)C-labeled tracers from natural counterparts in experimental sediments, FAME and alcohol-TMS-ether extracts from \(^{13}\)C-microalgae and from LIS bulk sediments were mixed according to a series of ratios (1:5, 1:1, 2:1, 4:1 and 6:1, in volume of extracts). Mass or concentration in the original extracts was quantified by previous GC analyses, so the mass ratio ($M_{13}/M_{12}$) of \(^{13}\)C-labeled to unlabeled compounds can be calculated in the mixed extracts. After the GC–MS run, the relative intensities of the characteristic fragments ($I_{74}$, $I_{76}$, $I_{87}$, and $I_{90}$ for 16:0 FAME; $I_{55}$, $I_{59}$, $I_{74}$, and $I_{76}$ for 16:1 and 18:1 FAMEs; and $I_{143}$ or $I_{147}$ for phytol-TMS-ether) were determined based on mass spectral measurement (peak areas). For phytol-TMS-ether, $m/z = 143$ and 147 are exclusive fragments for unlabeled and \(^{13}\)C-labeled compounds respectively. For FAMEs, the \(^{13}\)C-labeled compound (with a base peak at $m/z = 76$) usually had a small residue at $m/z = 74$ (probably due to a tiny amount of $^{13}$C in \(^{13}\)C-labeled microalgae), although the natural compound (base peak at $m/z = 74$) had a very weak ion fragment at $m/z = 76$. Therefore, when the intensity ratios of $^{13}$C to $^{12}$C from FAMEs ($I_{76}/I_{74}$) were estimated, a correction by [$I_{76}/(I_{74}-I_{76}/B)$] was made. The coefficient $B$ is the ratio ($I_{147}/I_{143}$) in the mass spectrum of the FAME extracted from the $^{13}$C-labeled microalgae. Similarly, the $^{13}$C-labeled 16:0 FAME had a residue at $m/z = 87$ while natural unlabeled 16:1 and 18:1 FAMEs had small residues at $m/z = 59$. All these residues were corrected using coefficients derived from pure unlabeled and labeled compounds.

Plotting of mass ratios ($M_{13}/M_{12}$) to intensity ratios ($R_i$) of characteristic fragments resulted in a good ($r^2 > 0.98$) linear correlation in all cases (Fig. 3). Based on this linear correlation and mass balance:

$$M_{13}/M_{12} = a(R_i) \pm b$$

$$M_{13} + M_{12} = M_T$$

where, $a$, $b$ are constant and $M_T$ is total mass of the labeled and unlabeled compounds, we can distinguish $^{13}$C compound from experimental sediments with labeled microalgae added. The formula is

$$M_{13} = M_T[a(R_i) \pm b]/[1 + a(R_i) \pm b]$$

Here, the intensity ratio ($R_i$) can be $I_{143}/I_{147}$ for phytol-TMS-ether or $[I_{76}/(I_{74}-I_{76}/B)]$, $[I_{59}/I_{55}/B]/I_{55}$, and $[I_{90}/(I_{87}-I_{90}/B)]$ for FAMEs. The negative or positive sign in Eq. (3) depends on the correlation of individual compounds. Although slopes of correlations derived from different intensity ratios (i.e. $I_{76}/I_{74}$ vs. $I_{90}/I_{55}$) were apparently different for one single compound, the relative standard deviations (RSD) for estimates of the $^{13}$C-labeled compound concentrations using two calibration lines were generally less than $\pm 3\%$. 

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3.3. Identification for $^{13}$C-labeled degradation products from microalgal incubations

In incubation experiments of $^{13}$C-microalgae in mixed sediments, two newly produced $^{13}$C-labeled compounds were identified based on mass spectra analysis. One is iso-15:0 fatty acid (Fig. 4), which has an apparent fragment (relative intensity may exceed 25%) at $m/z=76$ corresponding to an unlabeled one at $m/z=74$ in incubated sediments. For natural sediments, no fragment with $m/z=76$ occurs in the iso-15:0 fatty acid mass spectrum. The fragment with $m/z=76$ is generally too small (less than 1% of relative intensity) in the mass spectra of anteiso-15:0 and other short chain fatty acids (e.g. 14:0 and 15:0) to detect the occurrence of these labeled compounds. It is well known that iso- and anteiso-15:0

![Mass spectra for unlabeled, $^{13}$C-labeled, and mixed phytol-TMS-ether. The difference in $m/z$ of fragment $[\text{CH}_2\text{=CH}\text{-CH}_2\text{O-Si-(CH}_3)_3]^+$ between unlabeled and $^{13}$C-labeled phytol-TMS-ethers is used to distinguish the labeled compound from its natural sedimentary counterpart.](image-url)
Fatty acids commonly occur as a pair (similar level) in nature (Leo and Parker, 1966; Cooper and Blumer, 1968; Perry et al., 1979). In the original bulk sediments collected from LIS, the concentrations of iso-15:0 and anteiso-15:0 are roughly the same. The preferential production of 13C-labeled iso-15:0 relative to anteiso-15:0 is possibly a result of the microbial community’s domination by specific species in the experimental microcosms. For example, it was observed that the abundance of iso-15:0 was 10 times higher than that of anteiso-15:0 in the fatty acid composition of a sulphate-reducing bacterium (Parker and Taylor, 1983).

Another identified 13C-product is a normal C16 alcohol (Fig. 5), which has a fragment (base peak) at m/z = 315 corresponding to the unlabeled one at m/z = 299. This fragment in the C16 alcohol mass spectrum is M+ - methyl. At the starting point of incubation (t = 0 days), the mass spectrum of the C16 alcohol peak had no m/z = 315 fragment. This indicated that the occurrence of a m/z = 315 fragment in the mass spectrum of the C16 alcohol peak was a consequence of the degradation of 13C-labeled substrates. In fact, the distribution of fatty acids in natural LIS sediments was dominated by C20–C32 (even number) alcohols while the concentrations of short chain alcohols (C14–C18) were much less (unpublished data). During the incubations, concentration of C16 alcohol in the experimental sediments increased but other short chain alcohols showed no significant change.

Detailed analysis of the mass spectra of these two products can provide some insight into the formation mechanisms for these compounds. In the mass spectra of iso-15:0 fatty acid extracted from the incubated sediments, 13C fragments occurred at m/z = 76 and 90 along with unlabeled [CH2=O-H-O-CH3]+ (m/z = 74) and [CH2CH2(CO)-O-CH3]+ (m/z = 87). The absence of any significant occurrence of corresponding 13C-labeled M+ (m/z = 271) and M+ - 43 (m/z = 225) fragments in the mass spectra indicated that the newly produced iso-15:0 was partially 13C-labeled. The fragment at m/z = 76 is surely from 13C-labeled microalgae, although not all the carbon atoms in this newly produced molecule come strictly from a 13C source. As we know, iso-15:0 fatty acid is generally considered to be a bacteria-specific product.
compound (Volkman et al., 1980; Parker and Taylor, 1983), and its production during incubation is most likely linked to microbial processes. A higher yield of $^{13}$C-$\text{iso-15:0}$ was observed in the sediments without macrofauna (control cores) as compared to the sediments with macrofauna. This also implies that a microbial pathway leads to its formation (Sun et al., 2000). It can be inferred that bacteria biosynthesized this fatty acid via some small metabolites (C$_2$ or C$_3$ units) from both a $^{13}$C-labeled carbon source (added $^{13}$C-microalgae as substrate) and a natural unlabeled carbon source in bulk sediments. Rhead et al. (1972) reported a resynthesis pathway for palmitic acid based on incubation of $^3$H- and $^{14}$C-labeled oleic acid in sediments. The palmitic acid is resynthesized via acetyl-CoA (C$_2$ unit) which derived from the oxidation of oleic acid.

For C$_{16}$ alcohol-TMS-ether from incubated sediments, the $m/z$ difference between base peaks (M$^+$ - methyl) of

![Mass spectra for $\text{iso-15:0}$ fatty acid methyl ester in the Long Island Sound bulk sediment, incubated sediment ($t=0$ days), and incubated sediment ($t=5$ days). The occurrence of the $^{13}$C fragment ($m/z=76$) in the mass spectra indicates the production of $^{13}$C-labeled $\text{iso-15:0}$ during incubation.](image-url)
the unlabeled and the $^{13}$C-labeled compounds was 16 ($m/z = 315$–$m/z = 299$, Fig. 5). This indicates that almost all carbon atoms in the newly produced C$_{16}$ alcohol were labeled with $^{13}$C and the lost methyl was mainly from the TMS part. However, a fragment with $m/z = 314$ significantly occurred in the mass spectra, implying that the $^{13}$C-labeled methyl in the alcohol chain is possibly removed from the uniformly-$^{13}$C-labeled alcohol chain.

The mass spectrum suggests that the formation of $^{13}$C-labeled C$_{16}$ alcohol may be derived exclusively from $^{13}$C-algal substrate. In the experimental sediments, $^{13}$C-labeled microalgae was not the sole source of labile organic matter. If the $^{13}$C-labeled C$_{16}$ was produced through resynthesis of small metabolites, the labeling of $^{13}$C in the molecule should be partial rather than uniform. However, there were no significant occurrences of...
fragments between natural and uniformly 13C-labeled (M+ -15) ions in the mass spectra, implying that transformation from a 13C-labeled precursor may be the dominant pathway.

The transformation seems to be mediated by microbial processes. The first evidence of this is that more 13C-labeled C16 alcohol was produced in control cores (without macrofauna) than in cores with macrofauna. Even in the presence of macrofauna, a relatively higher concentration of 13C-labeled C16 alcohol was observed in 0.5–1 cm than in 0–0.5 cm sediment, where dissolved oxygen penetrates to ~0.4 cm (Sun et al.). Further evidence of this microbial mediation is that this 13C-alcohol did not occur in suspended particles ejected by Yoldia. Also, it is evident from the oxic/anoxic incubation that much more (~10×) 13C-labeled C16 alcohol was produced under anoxic conditions than under oxic conditions. The 13C-labeled microalgae do not contain C16 alcohol (confirmed by saponification of microalgae). It is not clear what the precursor of C16 alcohol is and which chemical reaction is involved. But, it was observed that the yield of 13C-labeled C16 alcohol was almost equivalent to the loss of 13C-phytol in the anoxic incubation of sediments spiked with 13C-microalgae (Sun et al. 2000). Thus, phytol seems to be a very possible precursor of this transformation product. Although the degradation pathway of phytol into several isopenoid compounds, including isopenoid alcohols, has been recognized (Volkman and Maxwell, 1984), no report has confirmed that the demethylation and hydrogenation of isopenoid phytol may result in a normal C16 alcohol. Considering the much higher (~5×) concentration of 13C-16:0 fatty acid than 13C-phytol present in the microalgae used in the experiments, it is possible that the newly-produced 13C-labeled C16 alcohol could be formed from 16:0 fatty acid through a reduction pathway. It is now well documented that reduction of lipids may occur in oxic environments, presumably due to the presence of anoxic microenvironments.

3.4. Comparison of degradation rate constants of 13C-labeled microalgal lipids

Since 16:1, 16:0, and 18:1 fatty acids and phytol are major lipid components in the experimental microalgae, they were chosen for determining the typical degradation rate constants of microalgal lipids. Table 1 lists the first-order degradation rate constants of 13C-microalgal lipids during three sets of incubations. For core incubations with and without the presence of Yoldia, the degradation rate constants were estimated based on the time-dependent variations of 13C-lipid inventories integrating the entire mixed layer (0–2 cm). For oxic/anoxic incubations of a thin layer of mixed sediments, the degradation rate constants were estimated by tracking the variations in concentration of 13C-lipids with time. The advantage of using 13C-tracers in this study is to differentiate microalgal lipid degradation from those of bulk sediments, including several other sources. For example, 16:0 fatty acid in natural sediments originates from phytoplankton, zooplankton, bacteria and benthic fauna (Parks and Taylor, 1983; Harvey et al., 1987; Volkman et al., 1989). In this study, degradation rate constants derived from 13C-lipids in labeled microalgae reflected the real reactivity of lipids found in natural microalgae.

Comparison between rate constants determined for cores containing animal to control cores demonstrated that the presence of benthic macrofauna (even one single species) would significantly enhance the degradation rate of microalgal lipids in sediments. The enhancement

<table>
<thead>
<tr>
<th>Experiment date</th>
<th>Treatments</th>
<th>Compounds</th>
<th>k-Control or anoxic</th>
<th>k-Animal or oxic</th>
<th>Ratios of k</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1996</td>
<td>A-I: control (no Yoldia)</td>
<td>16:1</td>
<td>0.016</td>
<td>0.046</td>
<td>2.88</td>
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<td></td>
<td>A-II: Yoldia (690/m²)</td>
<td>16:0</td>
<td>0.023</td>
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<td></td>
<td></td>
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<td>0.012</td>
<td>0.03</td>
<td>2.5</td>
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<tr>
<td></td>
<td></td>
<td>Phytol</td>
<td>0.025</td>
<td>0.078</td>
<td>3.12</td>
</tr>
<tr>
<td>March 1997</td>
<td>B-I: control (no Yoldia)</td>
<td>16:1</td>
<td>0.051</td>
<td>0.092–0.193</td>
<td>1.8–3.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16:0</td>
<td>0.062</td>
<td>0.106–0.23</td>
<td>1.71–3.83</td>
</tr>
<tr>
<td></td>
<td>B-II to B-IV: Yoldia (230–1160/m²)</td>
<td>18:1</td>
<td>0.048</td>
<td>0.06–0.131</td>
<td>1.25–2.73</td>
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<tr>
<td></td>
<td></td>
<td>Phytol</td>
<td>0.046</td>
<td>0.081–0.138</td>
<td>1.76–3</td>
</tr>
<tr>
<td>August 1997</td>
<td>C-I: anoxic</td>
<td>16:1</td>
<td>0.012</td>
<td>0.101</td>
<td>8.42</td>
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<td>0.107</td>
<td>21.4</td>
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<td>0.14</td>
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was due to changes in the decomposition regime by *Yoldia*, which has been discussed in considerable detail in another paper (Sun et al., 2000). A greater difference (∼10×) in rate constants of lipids was observed between oxic and anoxic incubations of 13C-labeled microalgae. When free molecules of fatty acids (i.e., 14C-labeled 16:0 and 18:1) were incubated in oxic and anoxic sediments, the differences in rate constants were much less (Sun et al., 1997). The molecular association in microalgal cells may be responsible for the greater difference between oxic and anoxic degradation of lipids. Most fatty acids in the 13C-labeled microalgae exist in phospholipids and galactolipids as membrane components, as confirmed by Si-gel column chromatographic separation (unpublished data). It is likely that aerobic heterotrophic organisms have a variety of enzymes allowing decomposition of lipid complexes in cells while anoxic decomposition of lipid complexes is hampered by inefficient and slow enzymatic hydrolysis (Canfield, 1994; Kristensen et al., 1995). Reichardt (1986) observed that sediment redox conditions affect enzyme activity in different ways.

4. Conclusions

Mass spectral characterization of 13C-labeled lipids contained in labeled microalgae provides a powerful approach for studying the degradation of planktonic organic matter in marine sediments. The distinction and quantification of 13C-labeled compounds from natural counterparts in the sediment matrix enable us to determine the decomposition rates of microalgal lipids. Identification of 13C-labeled products during the decomposition of organic matter and the detailed mass spectra analysis reveal that different pathways (resynthesis vs. transformation) can be used by microorganisms for altering algal organic matter. Estimates of degradation rate constants derived from 13C-tracers indicate that redox conditions and presence of aerobic macrofauna (e.g. *Yoldia*) apparently influence degradation rates of microalgal lipids in sediments.

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