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Isotopic compositions of lipid biomarker compounds in estuarine plants and surface sediments

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Abstract

We examined the isotopic compositions of fatty acids, sterols, and hydrocarbons isolated from three coastal macrophytes (*Zostera marina, Spartina alterniflora,* and *Juncus roemerianus*) in order to investigate the relative contribution of these vascular plants as sources of organic matter in coastal sediments such as Cape Lookout Bight, North Carolina. On average, lipid biomarker compounds extracted from the plants were depleted in ^{13}C by 3–5‰ relative to δ^{13}C total organic carbon (TOC). However, individual compounds within each lipid class varied by up to 5.6‰. Trends in the isotopic compositions of lipids were consistent with $\delta^{13}C_{TOC}$; compounds obtained from *Z. marina* were the most enriched in ^{13}C and those from *J. roemerianus* were the most depleted. The range in isotopic abundances and molecular compositions of the sediments was greater than that obtained from the plants, indicating that additional, presently unidentified sources of organic matter contribute to the Cape Lookout Bight sediments. Similarity between the signatures for suspended particulate matter and the sediments indicates that much of the sedimentary organic matter is derived from algal and bacterial sources. Bacterial sources of organic matter are likely greater during summer/early fall, and incorporation of ^{13}C-enriched bacterial biomass may contribute to observed seasonal shifts in $\delta^{13}C_{TOC}$ in the surficial sediments.

Organic matter preservation is greater in coastal sediments than for any other sedimentary reservoir (Romankevich 1984). Hence, information about processes controlling the delivery of organic matter to coastal sediments and how the signatures of these inputs are reflected in newly deposited sediments is important to our understanding of global biogeochemical cycles. However, variations in productivity, as well as fluctuations in delivery, make it difficult to resolve processes contributing to the enhanced storage of organic matter in coastal environments. Understanding the factors controlling the fluxes of organic matter to nearshore sediments is further complicated by the fact that natural organic matter originates from a diverse array of marine and terrigenous materials. Organic materials derived from these environmental end-members also lie along a continuum of reactivity ranging from more labile components dominant in the inputs from phytoplankton, benthic microalgae, and macroalgae to less reactive materials from marsh and sea grasses, higher plants, and soils.

Although the biochemical composition of these sources vary, differences in source signatures are not always unique enough to identify components in complex mixtures such as sediments. Traditionally, approaches for tracing inputs of organic matter have included elemental analysis (e.g. C : N ratios) and carbon and nitrogen stable isotopic composition (Fry et al. 1977; Sweeney and Kaplan 1980; Peterson et al. 1985). Detailed evaluations of the sources and reactivity of organic matter in coastal sediments have also made use of biomarker compounds covering a range of reactivities, including lignin oxidation products, lipids, and carbohydrates (Hedges and Parker 1976; Hedges et al. 1988; Venkatesan et al. 1988). Generally speaking, approaches that have used multiple techniques have been most successful in unraveling the identity of the sources of organic matter in complex nearshore sediments. Often, however, overlap in molecular or isotopic signatures has produced equivocal results.

Recent technological advances have increased our ability to determine the origins of sedimentary organic matter by the analysis of the stable isotopic compositions of individual compounds (Hayes et al. 1990; Freeman et al. 1990; Rieley et al. 1991). Measurement of the isotopic composition of individual compounds by isotope ratio-monitoring gas chro-

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matography–mass spectrometry (irm-GCMS) shows promise for resolving the origins of biological marker compounds (biomarkers). Biomarkers vary in their specificity to a given source and include compounds useful in identifying groups of organisms (e.g. algae vs. terrestrial vascular plants) to those useful in identifying organisms to the species or genus level. Given this range in biomarker specificity, isotopic information may be useful in resolving sources of organic matter, particularly when a given compound is synthesized by a number of organisms with isotopically distinct signatures. As the availability of this technique is relatively recent, our understanding of the isotopic composition of biomarker compounds derived from specific organisms is somewhat limited. Until now, these investigations have focused on vascular plants and trees (Rieley et al. 1991; Collister et al. 1994), marine microalgae (Popp et al. 1989; Freeman and Hayes 1992; Wakeham et al. 1993), or bacteria (Freeman et al. 1990, 1994; Freeman 1991; Prahl et al. 1992; Summons et al. 1994). The ability to interpret irm-GCMS data from both an ecological and geological perspective requires an increased understanding of the isotopic compositions of biological source materials at the molecular level. In addition, an understanding of the processes occurring during organic matter production and early diagenesis and how these processes influence the incorporation of biomarker signatures into the sedimentary record is also necessary. For example, isotopic signatures of compounds from the same organisms can vary if the isotopic composition of the substrate, isotopic fractionation during assimilation, and the relative abundances of lipids change during the growth cycle (Summons et al. 1994).

In this paper, we present results from a study that examined the carbon isotopic composition ($\delta^{13}C$) of individual molecules from three classes of lipid biomarker compounds obtained from some common coastal plants (*Spartina alterniflora, Zostera marina,* and *Juncus roemerianus*). These plants were selected both because of their wide geographic distribution and because they are thought to contribute significantly to the primary production of nearshore systems. Thus, identifying unique molecular and isotopic signatures could provide a useful tool for evaluating the role of these plants in food webs and for quantifying their contribution to organic matter accumulation in coastal sediments. The goals in this study were (1) to examine the isotopic composition of biomarker compounds of a few species of important macrophytes using compound-specific isotope analysis, (2) to examine isotopic variation in these source plants by comparing the isotopic composition of biomarker compounds derived from multiple lipid classes, and (3) to evaluate how these macrophytes contribute to the organic content of a coastal sediment with nearby marsh and seagrass habitats. To address the third component of the study, we applied irm-GCMS technology to the analysis of suspended particles and surficial sediments collected from Cape Lookout Bight, North Carolina (CLB). For this initial study, we selected compound classes abundant in the plants and sediments and routinely analyzed in sediments by other investigators.

Materials and methods

Study site—CLB is a small coastal basin located at the southern end of the Outer Banks of North Carolina (34°37′N, 76°33′W). Detailed study of this system has documented that on an annual basis, accumulation of sediment and organic matter at this site has been at steady state for the past two decades (Chanton et al. 1983; Haddad and Martens 1987; Canuel et al. 1990). Rates of carbon remineralization are also at steady state on annual time scales (Martens et al. 1992; Martens and Klump 1984); however, on shorter time scales accumulation of sediment and organic matter composition is variable (Canuel et al. 1990; Canuel and Martens 1993). Sediment accumulation occurs primarily during winter and spring months, and inputs of organic matter associated with these events are primarily derived from a mixture of sources originating in the backbarrier island lagoons and marshes (Haddad and Martens 1987; Haddad et al. 1992; Canuel and Martens 1993). A large fraction (71%) of the carbon buried at this site is highly reworked organic matter, primarily of algal origin, that has been recycled in surrounding coastal environments for hundreds of years, giving radiocarbon ages of organic carbon in the upper meter of sediment ranging from 500 to 1,000 yr in age (Martens et al. 1992). During summer when rates of sediment accumulation are negligible, high rates of bacterially mediated remineralization alter the composition of the sedimentary organic carbon by metabolizing a fraction of the organic matter delivered during winter/spring depositional events (Canuel and Martens 1993).

Sample collection and analysis—Healthy, green leaves without visible signs of epiphytes were collected from marshes and seagrass beds proximal to the CLB site during late spring and early summer. For this study, we focused on plants dominant in the salt marshes and intertidal areas along the shoreline of North Carolina and throughout the Southeast. For example, in the Newport River Estuary, located near CLB, Thayer et al. (1978) estimated that seagrass meadows dominated by *Z. marina* and *Halodule wrightii* contribute 47–65% of the total primary production of the back barrier island lagoon systems. In this same system, *S. alterniflora* and phytoplankton contribute 28–43% and 8–10%, respectively, of the primary production. In addition, we selected plants with differing biosynthetic pathways and CO_2 sources: *Z. marina* (C_3 plant; but like other aquatic plants, its isotopic signatures are similar to C_4; Benedict et al. 1980), *S. alterniflora* (C_4 salt marsh plant), and *J. roemerianus* (C_3 freshwater marsh plant). Leaves (several grams dry weight) for each species were collected from several plants (10–20) growing at a single site within an area encompassing several square meters. In our investigation, we focused on the leaves because they comprise a large fraction of the export from marshes and seagrass beds (Bach et al. 1986). We point out that while other investigators have noted that the bulk isotopic signatures of other components of the plants (e.g. roots and rhizomes) may differ from the leaves (Deines 1980), others have not (Benner et al. 1987; Fogel et al. 1989). Although few data on intramolecular $\delta^{13}C$ values of different plant tissues are available, isotopic signatures of amino acid fractions from roots and leaves of *S. alterniflora* indicate that these compartments can vary dramatically (N. Blair pers. comm.). Thus, the results reported herein may not be applicable to other tissues of the plants or representative of the whole plants. For this initial study, the goal was to collect plant samples typical of periods of high productivity. Interplant or seasonal variability within each site was not examined.

Following collection, plant samples were first rinsed in tap water (followed by distilled water) to remove attached sediments and salt. The samples were then soaked in dilute HCl (5% by vol) to remove any calcium carbonate, followed by additional rinses with distilled water. A representative portion of the plant tissue samples was removed, dried, and ground with a mortar and pestle for bulk isotopic analysis. The remaining plant tissue was extracted with organic solvents as described below.

In addition, surface-water samples were collected from CLB and filtered. Suspended particles were collected on precombusted (450°C) 142-mm glass-fiber filters (Gelman A/E), placed in precombusted jars, and frozen until analysis. Sediment cores were collected using scuba and upon return to the laboratory, the cores were extruded and sectioned. For this study, only the surface sediment (0–0.5 cm), representing accumulation within a month of sample collection (Canuel et al. 1990; Canuel and Martens 1993), was analyzed.

Sediments and suspended particulate matter (SPM) were extracted with organic solvents (methylene chloride : methanol; 2 : 1, by vol) and processed as described in Canuel and Martens (1993). Hydrocarbons, sterols (as trimethylsilyl ethers), and fatty acids (as methyl esters) were analyzed by gas chromatography on a dimethylphenylsilicone fused silica capillary column (DB-5; J&W Scientific). Individual peaks were quantified relative to internal standards added just prior to analysis. Compounds were identified by relative retention times and structures were verified using combined GCMS (Hewlett-Packard 5890 gas chromatograph interfaced with a Finnigan Incos 50 mass spectrometer data system). Samples were run in the electron impact mode and spectra collected over the mass range 50–550.

Isotope analysis of bulk carbon—Prior to elemental analysis, carbonate was removed from the sediment and suspended particle samples with HCl following methods detailed in Canuel and Martens (1993). Elemental analysis (organic carbon, total nitrogen) was performed using a Carlo Erba NA 1500 elemental analyzer, and the CO_2 resulting from the combustion of organic carbon was collected. The isotopic composition ($\delta^{13}C$ of total organic carbon, $\delta^{13}C_{TOC}$) of the collected CO_2 was analyzed using a Finnigan MAT 251 stable-isotope mass spectrometer. Isotopic values are presented in the δ notation in parts per million (‰) deviations and expressed relative to the PDB standard. Precision of the $\delta^{13}C_{TOC}$ analyses was ±0.20‰. When available, the isotopic composition ($\delta^{13}C$) of a portion of the total lipid extract (TLE) was also determined. An aliquot of TLE was placed in a pyrex tube and dried gently under dry N_2. An excess of cupric oxide was added, and the tube was evacuated and sealed. The TLE was combusted at 500°C for 12 h, and the resulting CO_2 was purified by cryogenic distillation. Isotopic abundances were determined using a Finnigan MAT 252 stable-isotope mass spectrometer by way of a conventional dual-inlet system.

Compound-specific isotope analyses—Compound-specific isotope analyses were determined using a GC-combustion system connected to a stable-isotope mass spectrometer, as described by Freeman and Wakeham (1992). Derivatives of both the organic acids and sterols were made in order to improve their chromatographic behavior. The $\delta^{13}C$ of carbon in the added trimethylsilane group (for sterols) and the methyl ester (for fatty acids) was determined by mass balance (Abrajano et al. 1994) using androstanol and heneicosanoic acid, respectively, as isotopic standards. Precision for these analyses is typically ≥0.5‰; larger errors generally reflect trace contributions from partially co-eluting compounds. All isotopic compositions are expressed relative to the primary standard, PDB: $\delta^{13}C = (^{13}R_{SA}/^{13}R_{PDB} - 1) \times 10^3$, where $^{13}R_{SA}$ represents the $^{13}C : ^{12}C$ abundance ratio for the sample and $^{13}R_{PDB} = 0.0112372$.

Results

Bulk carbon-isotope analyses—In a previous investigation where the $\delta^{13}C_{TOC}$ associated with surficial sediments collected from CLB was determined on a monthly basis (Canuel and Martens 1993), $\delta^{13}C_{TOC}$ ranged from −17.8 to −20.3‰. Samples with the most extreme values were collected in February and October 1988; hence, these were chosen for the detailed molecular-level analyses presented here. In conjunction with the isotopic analyses of lipid biomarker compounds, we also determined the $\delta^{13}C$ of the TLE for the surficial sediments. The $\delta^{13}C_{TLE}$ values for the surficial sediments ranged from −20.8 to −22.3‰ and were generally depleted in ^{13}C by 1–3‰ relative to TOC (E. Canuel unpub. data). These values are in good agreement with previous measurements of fatty acid (−22.1 ± 0.5‰) and neutral lipid fractions (−22.9 ± 0.3‰) for CLB sediments (Blair and Carter 1992). TOC associated with SPM collected from CLB in August 1991 had a $\delta^{13}C$ value of −18.4‰, similar to previously published values for this region (Thayer et al. 1978; Currin et al. 1995).

The stable-isotope analysis of the TOC yielded values expected for the C_3 and C_4 plants selected for this study (Haines 1976; Thayer et al. 1978; Fogel et al. 1989). Although sea grasses are C_3 plants, they have $\delta^{13}C_{TOC}$ values similar to C_4 plants because (1) CO_2 dissolved in seawater rather than atmospheric CO_2 is used as the inorganic carbon source, and (2) diffusional exchange of CO_2 across the cell membrane is rate limiting; thus, the carboxylation step does not express its isotope effect (Benedict et al. 1980; O'Leary 1981; Grice et al. 1996). The seagrass *Z. marina* was the most enriched in ^{13}C (−10‰); the marsh grasses *J. roemerianus* and *S. alterniflora* had $\delta^{13}C_{TOC}$ of −26 and −12.6‰, respectively (Table 1). Sufficient material to measure the $\delta^{13}C$ of the TLE was available for only one of the plants, *Z. marina*. Its $\delta^{13}C_{TLE}$ was −15 (±0.6)‰, depleted in ^{13}C by 5‰ relative to bulk carbon (Table 1).

Sterol compositions—Each of the three plants had simple sterol compositions dominated by 24-ethylcholest-5-en-3β-

Table 1. Summary of $\delta^{13}C$ (‰) values (means ± SD).

	Juncus	*Spartina*	*Zostera*	SPM (13 Sep 91)	Sediments (27 Feb 88)	Sediments (2 Oct 88)
Total organic carbon (TOC)	−26.0	−12.6	−10.0	−18.4 (0.1)	−20.3 (0.3)	−17.8
Total lipid extract (TLE)	n.a.*	n.a.	−15.2 (0.6)	n.a.	−21.5 (0.1)	n.a.
Fatty acids (FAs)†	−32.8 (1.6)	−18.4 (2.3)	−14.8 (2.9)	−23.2 (2.5)	−25.3 (5.4)	−19.4 (11.0)
Sterols†	−29.0 (0.4)	−19.0 (4.3)	−15.0 (0.5)	−25.3 (2.9)	−22.9 (2.8)	−20.4 (1.3)
Hydrocarbons (HCs)†	−33.8 (1.8)	−22.6 (2.0)	−18.9 (2.4)	−28.3 (3.6)	−28.4 (4.7)	−28.8 (4.8)
$\Delta\delta^{13}C$: TOC,						
Lipid			5.2		1.2	
FAs	6.8	5.3	4.8	4.8	5.0	1.6
Sterols	3.0	6.4	5.0	6.9	2.6	2.6
HCs	7.8	10.0	8.9	9.9	8.1	11.0

* Lipid extract was not available (n.a.) for $\delta^{13}C$ analysis.

† Averaged over the entire carbon-number range present.

ol (29Δ5; Fig. 1A). The isotopic composition of sterol components ranged from −14 to −17‰ for *Z. marina*, −28 to −30‰ for *J. roemerianus*, and −17 to −21‰ for *S. alterniflora*. On average, the plant sterols were depleted in ^{13}C relative to TOC by 3–6‰ (Table 1). The SPM and sediment samples contained a more varied assemblage of sterols, suggesting a mixture of sources other than the marsh plants, and were dominated by contributions from phytoplankton (Fig. 1B). The isotopic signature of sterols associated with suspended particles collected from the water column indicates two isotopically distinct sources: one that produces C_{28} and C_{29} sterols (e.g. 24-methylcholesta-5,22-dien-3β-ol [28Δ5,22], 24-ethylcholesta-5,22-dien-3β-ol [29Δ5,22], 24-ethylcholest-5-en-3β-ol [29Δ5], and 24-ethylcholesta-5,24(28)-dien-3β-ol [29Δ5,24(28)]), typical of vascular plants (Huang and Meinschein 1979), with $\delta^{13}C$ values of −26 to −27‰. A second source produces cholest-5-en-3β-ol [27Δ5] and 24-methylcholesta-5,24(28)-dien-3β-ol [28Δ5, 24(28)], typical of diatoms or cyanobacteria (Volkman 1986), with $\delta^{13}C$ values of −22 to −23‰ (Fry and Wainwright 1991; Raven and Johnston 1991; Freeman et al. 1994). With the exception of the C_{29} sterols (Δ^5 and $\Delta^{5,22}$), the isotopic signatures of most sterols in the surface sediments were similar to those obtained for SPM from the overlying waters (Fig. 1B). In contrast, the isotopic signatures of $C_{29}\Delta^5$ and $C_{29}\Delta^{5,22}$ sterols in the sediment did not match those obtained from either the SPM or plants, although the isotopic compositions of these sediment sterols were fairly similar to *S. alterniflora*. Like the TOC, total sterols and individual sterols associated with the sediments collected in October 1988 were generally enriched in ^{13}C relative to sterols in sediments collected in February (Table 1, Fig. 2).

Fatty acids—The plant samples were characterized by fatty acid distributions dominated by 16:0 and di- and triunsaturated C_{18} fatty acids (Fig. 3A). Mean isotopic signatures of the fatty acids were similar to those of the sterols (Table 1). Like the sterols, the diversity of fatty acids in the SPM and sediment samples was greater than in the plants (Fig. 3B), again indicating that organic matter was derived from a complex mixture of sources. Fatty acids in the SPM sample were typical of, although not unique, to diatoms, with 14:0, 16:0, and 16:1 being the major components. Lower concentrations of components exclusive to phytoplankton (i.e. polyunsaturated C_{20} and C_{22} fatty acids; Volkman et al. 1989) were also present in the SPM and the isotopic signatures of these carboxylic acids ranged from −19 to −22‰ (Fig. 3B). Isotopic ratios of fatty acids in the surficial sediments ranged from −16 to −22.5‰ in February and from −13 to −25‰ in October. Lower molecular weight fatty acids ($<C_{18}$) collected in February were depleted in ^{13}C relative to those in sediments collected in October (Table 2). In both sediment samples, bacterial fatty acids (15:0 and 17:1 + 17:0; Johns et al. 1977) were enriched in ^{13}C relative to other fatty acids (Fig. 2). In particular, bacterial fatty acids in the October sediments were more enriched in ^{13}C than all other compounds examined in this study (−13 to −14‰). Unfortunately, we were unable to obtain δ-values for other bacteria-specific compounds (e.g. *iso*- and *anteiso*-branched C_{15} and C_{17} acids).

Hydrocarbons—In contrast to the the sterol and fatty acid distributions that were similar for the three plants, the three species of marsh and seagrasses had distinct hydrocarbon compositions (Fig. 4A). *Z. marina* and *S. alterniflora* contained a homologous series of *n*-alkanes with a predominance of the odd-numbered compounds. The *n*-alkanes of *S. alterniflora* were in the C_{23}–C_{33} range, maximizing at C_{29}, whereas the carbon chain lengths for *Z. marina* were in the C_{17}–C_{27} range, with a maximum at C_{21}. *J. roemerianus*, on the other hand, contained a hydrocarbon distribution different from the other two plants and unusual for higher plants in general (Fig. 5). Hydrocarbons isolated from *J. roemerianus* were dominated by homologues in the C_{21}–C_{33} range and contained pairs of saturated *n*-alkanes and monounsaturated alkenes (monoenes); the alkanes maximized at C_{27} and the monoenes at C_{28}. For the odd-numbered compounds the alkanes dominated, whereas monoenes were more abundant for the even-numbered compounds (Fig. 5). Alkane–alkene pairs were not adequately resolved in irm-GCMS; thus, isotopic values (Fig. 4A) presented are for both compounds. Isotopic signatures for hydrocarbons from each of the plants overlapped with those obtained for the sterols and fatty acids, although they were generally more depleted in ^{13}C than the other compound classes (Table 1). Isotopic

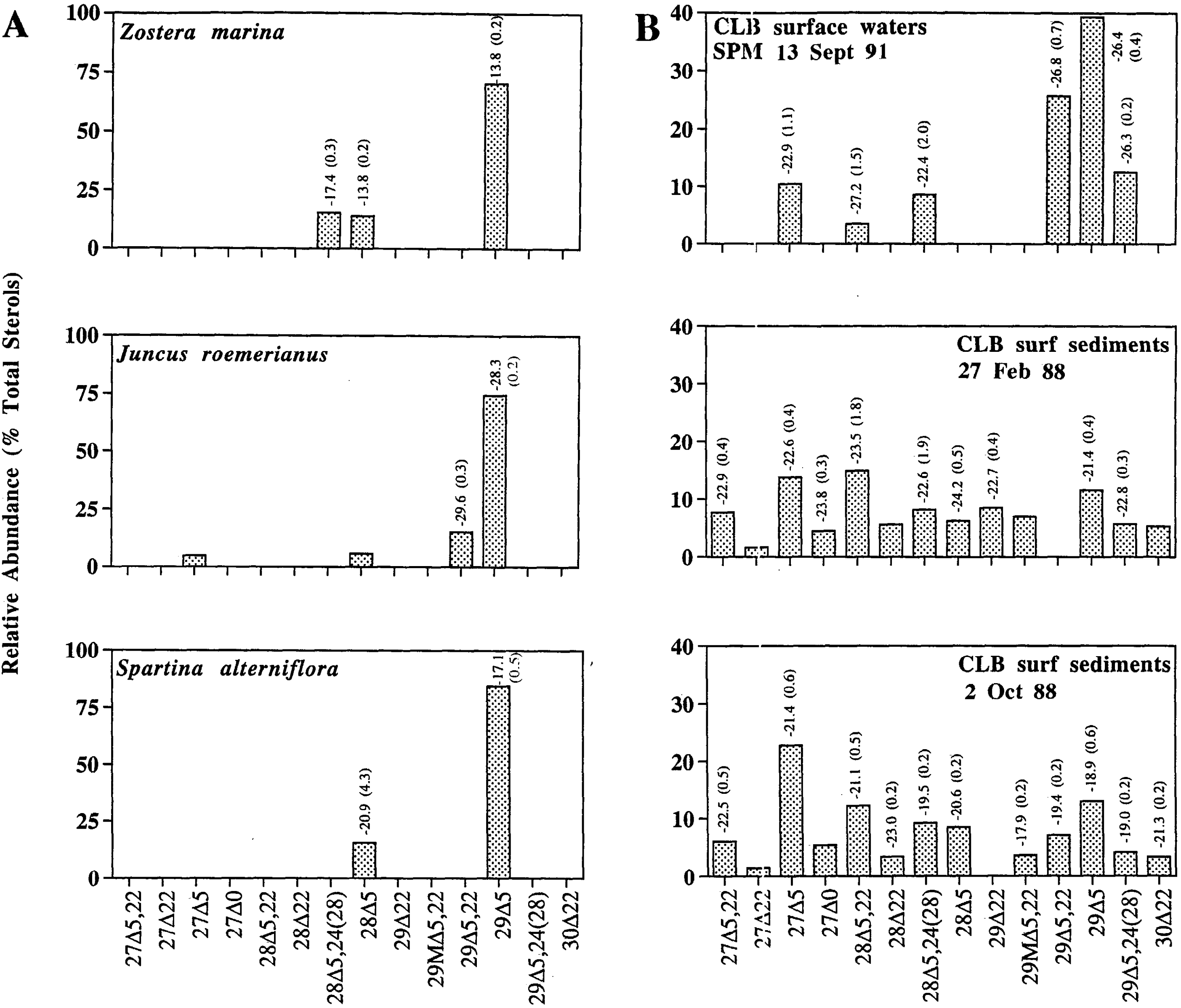

Fig. 1. Histograms showing the relative abundance (% total concentration) of sterols isolated from sea and marsh grasses (A), as well as suspended particulate matter (SPM) and surficial sediments (0–0.5 cm) collected from Cape Lookout Bight (CLB), North Carolina (B). Isotopic abundances ($\delta^{13}C$, per mil) for individual compounds (±SD) are listed above each bar. Sterol designations are cholest-5,22-dien-3β-ol (27Δ5,22), cholest-22-en-3β-ol (27Δ22), cholest-5-en-3β-ol (27Δ5), 5α(H)-cholestan-3β-ol (27Δ0), 24-methylcholest-5,22-dien-3β-ol (28Δ5,22), 24-methylcholesta-5,24(28)-dien-3β-ol (28Δ5,24[28]), 24-methylcholest-5-en-3β-ol (28Δ5), 24-ethylcholest-22-en-3β-ol (29Δ22), 23,24-dimethylcholest-5,22-dien-3β-ol (29MΔ5,22), 24-ethylcholesta-5,22-dien-3β-ol (29Δ5,22), 24-ethylcholest-5-en-3β-ol (29Δ5), 24-ethylcholesta-5,24(28)-dien-3β-ol (29Δ5,24[28]), and 4,23,24-trimethylcholest-22-en-3β-ol (30Δ22). Total sterol concentrations in the surface sediments were 40.8 and 16.5 $\mu g\ g^{-1}$ dry wt sediment, respectively, for the February and October sediment samples (Canuel and Martens 1993).

abundances for individual hydrocarbons ranged from −16 to −20‰ for *Z. marina*, −32 to −34‰ for *J. roemerianus*, and −21 to −27‰ for *S. alterniflora*.

The SPM sample contained high abundances of C_{25} highly branched isoprenoids (HBI) with two to four double bonds (Fig. 4B). Because these components could not be resolved adequately for the isotopic measurements, the $\delta^{13}C$ values listed are for all C_{25} HBI alkenes. In the SPM, >50% of the hydrocarbon abundance is comprised of these C_{25} HBI, with the dominant structures being two isomers of 25:3. These compounds are likely derived from diatoms (Rowland and Robson 1990; Volkman et al. 1994), as is supported by their enrichment in ^{13}C (Freeman et al. 1994). The SPM sample also contained low concentrations of *n*-alkanes with a slight odd-numbered carbon predominance at higher molecular weights. Isotopic compositions of these *n*-alkanes showed

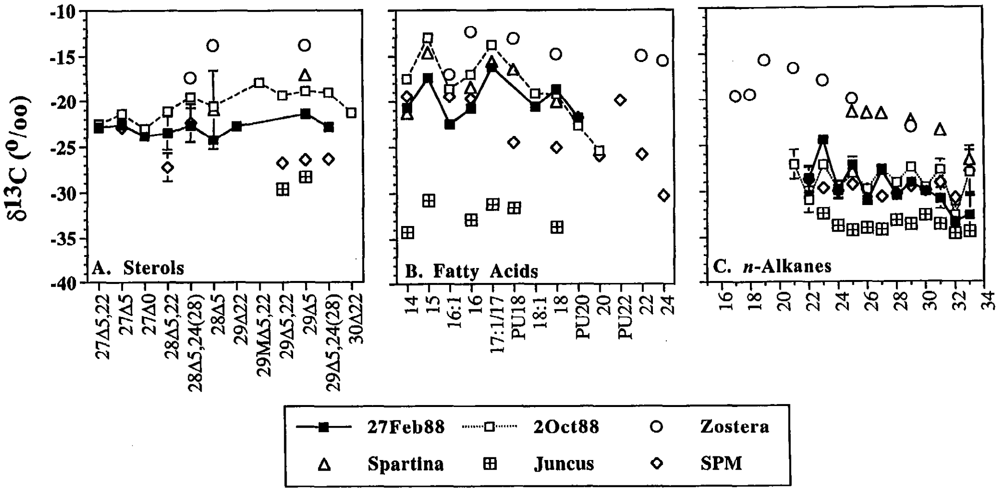

Fig. 2. Comparison of isotopic abundances for individual compounds from three classes of lipid compounds, sterols (A), fatty acids (B), and *n*-alkanes (C). In cases where the error bars are not visible, the error is less than the size of the symbol. *See* Fig. 1 for sterol designations.

little variation (−28 to −31‰), indicating possible contamination from a petroleum source, consistent with the compound distributions.

In the sediments, there was a homologous series of *n*-alkanes with an odd carbon number predominance over the range C_{23}–C_{35} in February and C_{17}–C_{35} in October (Fig. 4B). In February, the distribution maximized at C_{29}, whereas there was a bimodal distribution in October with maxima at C_{25} and C_{29}. Although C_{25} HBI were present in both sediment samples, their relative abundance was greater in February. As in the SPM, the C_{25} HBI were isotopically enriched in ^{13}C relative to the *n*-alkanes. Similar to the other compound classes, we find a range in the isotopic compositions of the hydrocarbons. Even-numbered *n*-alkanes were generally depleted in ^{13}C by 1–2‰ relative the odd-numbered ones (Figs. 2, 4B) which has been shown by other investigators (Rieley et al. 1991; Collister et al. 1994; Freeman et al. 1994). Phytane was the most ^{13}C-depleted compound in the October sediment. On the whole, the isotopic compositions of hydrocarbon compounds were similar in the sediments collected during February and October, although the *n*-C_{29} and *n*-C_{31} alkanes were isotopically depleted in the February vs. October sediment samples.

Discussion

Isotopic composition of plants—There have been relatively few studies where the isotopic compositions of molecular components of individual organisms have been determined, and of these studies, fewer examined the composition of components in multiple compound classes from a single organism. Previous investigations of higher plants (Rieley et al. 1991; Collister et al. 1994) and bacteria (Blair et al. 1985; Summons et al. 1994) have shown that isotopic variations within and between compound classes can be substantial (up to 8‰), indicating the necessity for understanding the processes contributing to these variations before we can use compound-specific isotope analyses to interpret reliably the sedimentary record. Similarly, in addition to the expected differences in the isotopic signatures between the plants resulting from their differing biosynthetic pathways (C_3 vs. C_4), we found considerable variation in the isotopic composition of biomarker compounds isolated from each of the seagrass and marsh plants (Table 1, Fig. 2). Compounds within each of the lipid classes varied in isotopic abundance by up to 7‰. Subgroups within compound classes also showed distinct isotopic compositions. For example, isotopic signatures for saturated, monounsaturated, and polyunsaturated fatty acids in each of the plants differed (Table 2). In some cases, this difference was several per mil, although variations were not consistent between compound type (Table 2). *n*-Alkanes and, to some extent, fatty acids isolated from *Z. marina* and *S. alterniflora* also exhibited increased depletions in ^{13}C as carbon chain-length increased (Fig. 2).

Within a single plant, isotope effects associated with biosynthetic pathways such as chain elongation and desaturation contribute to isotopic variations within and between compound classes (Collister et al. 1994). During lipid synthesis, for example, the carboxyl carbon in acetate becomes depleted in ^{13}C due to decarboxylation (DeNiro and Epstein 1977; Monson and Hayes 1980, 1982*a,b*) as well as other metabolic processes (Blair et al. 1985). Thus, variations in the $\delta^{13}C$ of compounds and compound classes among plants can be understood in terms of the relative flows of lipid precursor compounds (such as pyruvate) to other metabolic intermediates. In this study, the wide range in the isotopic compositions of individual hydrocarbons indicates that differences between hydrocarbons and other compound classes are not statistically significant (Table 1), although previous studies have suggested that, in general, *n*-alkyl lipids would be depleted in ^{13}C relative to polyisoprenoid compounds (reviewed in Hayes 1993). In higher plants, *n*-alkanes are the product of chain elongation and subsequent decarboxylation of precursor fatty acids but are ultimately comprised of carbon derived from acetate. Sterols and other polyisoprenoids are constructed from repeating isoprene units biosynthesized

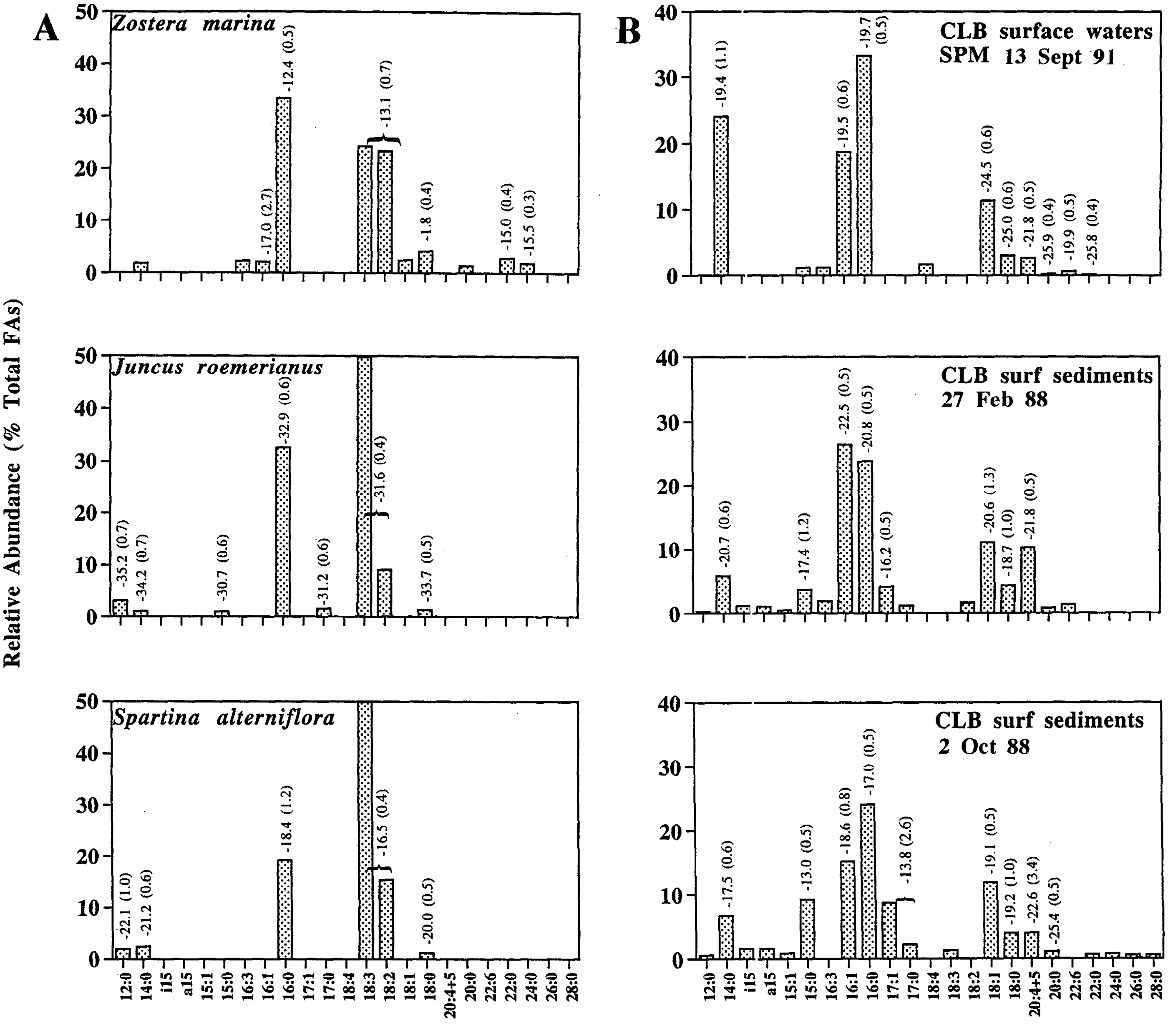

Fig. 3. Relative abundances (% total concentration) of fatty acids (FAs) from plants (A), as well as CLB SPM and surface sediments (B). Isotopic abundances ($\delta^{13}C$, per mil) for individual compounds (±SD) are listed above each bar. In the surface sediments collected during February and October, total fatty acid concentrations were 114 and 203 $\mu g\ g^{-1}$ dry wt sediment, respectively (Canuel and Martens 1993). In some cases, peaks could not be resolved for isotopic measurements (e.g. 17:1 and 17:0 have been combined, and 18:1 includes an unresolved mixture of the Δ^9 and Δ^{11} isomers).

from the decarboxylation of mevalonic acid, a product of three acetate units. Polyisoprenoids are thus expected to be enriched in ^{13}C relative to *n*-alkyl lipids due to the smaller contribution of the isotopically depleted carboxyl carbon from acetate; the ratio of methyl to carboxyl carbon in polyisoprenoids is 3:2 and is 1:1 in *n*-alkanes.

Consistent with these predictions, we find similarity in the isotopic compositions of *n*-alkanes and even-numbered *n*-alkanoic acids, from which they are presumably derived in the marsh grasses *J. roemerianus* and *S. alterniflora*. In contrast, saturated, even-numbered fatty acids and *n*-alkanes obtained from *Z. marina* were markedly different in their isotopic composition. These results, as well as those from other investigators, point to the need to consider within- and between-class variations in isotopic abundance when assigning sources to sedimentary organic matter. Clearly, there are complexities in biosynthetic pathways that limit our ability to predict the isotopic abundance of individual compounds based on the bulk ($\delta^{13}C_{TOC}$) information alone.

Sources of organic matter: Marsh and seagrasses—Evidence for ^{13}C enrichment is sedimentary organic matter dur-

Table 2. Variation in fatty acid $\delta^{13}C$ values (means ± SD, expressed as per mil relative to PDB) (ND, no data).

	Saturated (even-numbered)	Saturated (odd-numbered)	Mono-unsaturated	Poly-unsaturated
J. roemernianus	−33.2 (1.3)	−30.9 (0.8)	ND	−31.6 (0.5)
S. alterniflora	−20.5 (1.7)	−15.1 (1.5)	ND	−16.5 (0.4)
Z. marina	−14.8 (0.9)	ND	−17.0 (2.7)	−13.1 (0.7)
SPM (9/13/91)	−24.3 (2.2)	ND	−22.0 (0.8)	−20.8 (0.7)
	$<C_{18}$: −19.6 (1.2)		$<C_{18}$: −19.5 (0.6)	
	$\geq C_{18}$: −26.7 (1.9)		$\geq C_{18}$: −24.5 (0.6)	
Sediments				
27 Feb 88	−20.0 (1.2)	−16.8 (0.8)	−21.5 (1.3)	−21.8 (1.5)
	$<C_{18}$: −20.7 (0.04)			
	$\geq C_{18}$: −18.7 (1.0)			
2 Oct 88	−19.8 (3.8)	−13.4 (0.5)	−18.9 (0.3)	−22.6 (3.4)
	$<C_{18}$: −17.3 (0.3)			
	$\geq C_{18}$: −22.3 (4.3)			

ing late summer and fall months relative to winter and spring months was obtained during a previous study where the $\delta^{13}C_{TOC}$ of the surficial sediments was determined on a monthly basis (Canuel and Martens 1993). These results led to our original hypothesis that variation in the isotopic signature of surficial sediments resulted from seasonal changes in the sources of organic carbon delivered to the study site. An objective in this study was to examine whether increased inputs of marsh and seagrass detritus enriched in ^{13}C account for the enrichment in ^{13}C found in the surface sediment TOC. Seasonality in the export of *Z. marina*, macroalgae, and *S. alterniflora* has been observed for seagrass beds in the vicinity of our CLB study site (Bach et al. 1986). These investigators found that export of *Z. marina* was highest in July and August; *S. alterniflora* export maximized in fall (October–January) and spring.

Although the timing for these export events is coincident with the enrichment in ^{13}C we find in CLB sediments (i.e. greater enrichment in ^{13}C in TOC collected in October vs. February), results from this study indicate that organic matter derived from these plants is not a dominant source of sedimentary carbon (Fig. 2). It is possible that this observation results from our choice of compound classes that are perhaps not the most diagnostic for these plants (e.g. hydroxy acids may provide better information; de Leeuw et al. 1995). It is also possible that the higher plant signatures may be more apparent in the bound phases than in the extractable phase analyzed in this study. For the compound classes analyzed in this study, however, we find that the lipid composition of the surficial sediments is much more diverse than that found in the plants, suggesting that sources other than the plants are also important. Moreover, the major biomarkers in the plants were not dominant in the extractable lipids obtained from these surface-sediment samples. Although diagenetic loss of the C_{18} polyunsaturated acids could explain their absence in the sediment samples, it is unlikely that this could account for the paucity of the macrophyte biomarkers. The compound distributions, as well as the isotopic signatures of the compounds, indicate that SPM from the overlying waters is a more likely source. A water-column origin for a portion of the organic matter is further supported by the fact that several of the compounds most abundant in the surficial sediments are known to be common to phytoplankton, particularly diatoms (e.g. 24-methylcholesta-5,22-dien-3β-ol [28Δ5,22], the C_{25} HBI, and 14:0, 16:1, and 16:0 fatty acids). Although the sediments are dominated by biomarker signatures that seem to originate from water-column phytoplankton, the isotopic signatures of compounds such as 16:0 and 24-ethylcholest-5-en-3β-ol (29Δ5), which are abundant in the marsh and seagrass plants, indicate that only a minor component of the sedimentary organic matter may be derived from *S. alterniflora*. Minor contributions from higher plants are consistent with previous results obtained using lignin oxidation products, which indicated that vascular plants contributed 23 ± 17% of the total carbon accumulating in CLB sediments over the past decade (Haddad and Martens 1987). Although our results do not provide us with conclusive information as to the likely sources for the vascular plant-derived component of extractable organic matter from CLB sediments, the irm-GCMS results do allow us to exclude *J. roemerianus* as a potential contributor to the lipid pool and suggest minimal inputs from *Z. marina*.

Sources of organic matter: Phytoplankton—Given our ability to exclude variations in the delivery of seagrass- and marsh grass-derived lipids (this study) and other materials (Haddad and Martens 1987) as the dominant mechanism for the isotopic enrichment of lipids found in the surficial sediments collected in October, we conclude that other sources and(or) diagenetic processes are responsible for these fluctuations. On the whole, the isotopic and molecular information indicates that phytoplankton is a likely source for a portion of the organic matter deposited in the sediments of CLB. As seasonal changes in environmental and(or) physiological factors may alter the isotopic composition of photosynthetic organisms, it is possible that the delivery of phytoplankton-derived organic matter to CLB sediments might remain constant, while the isotopic signature of that source might vary. Processes that could contribute to isotopic variation in phytoplankton might include changes in the source

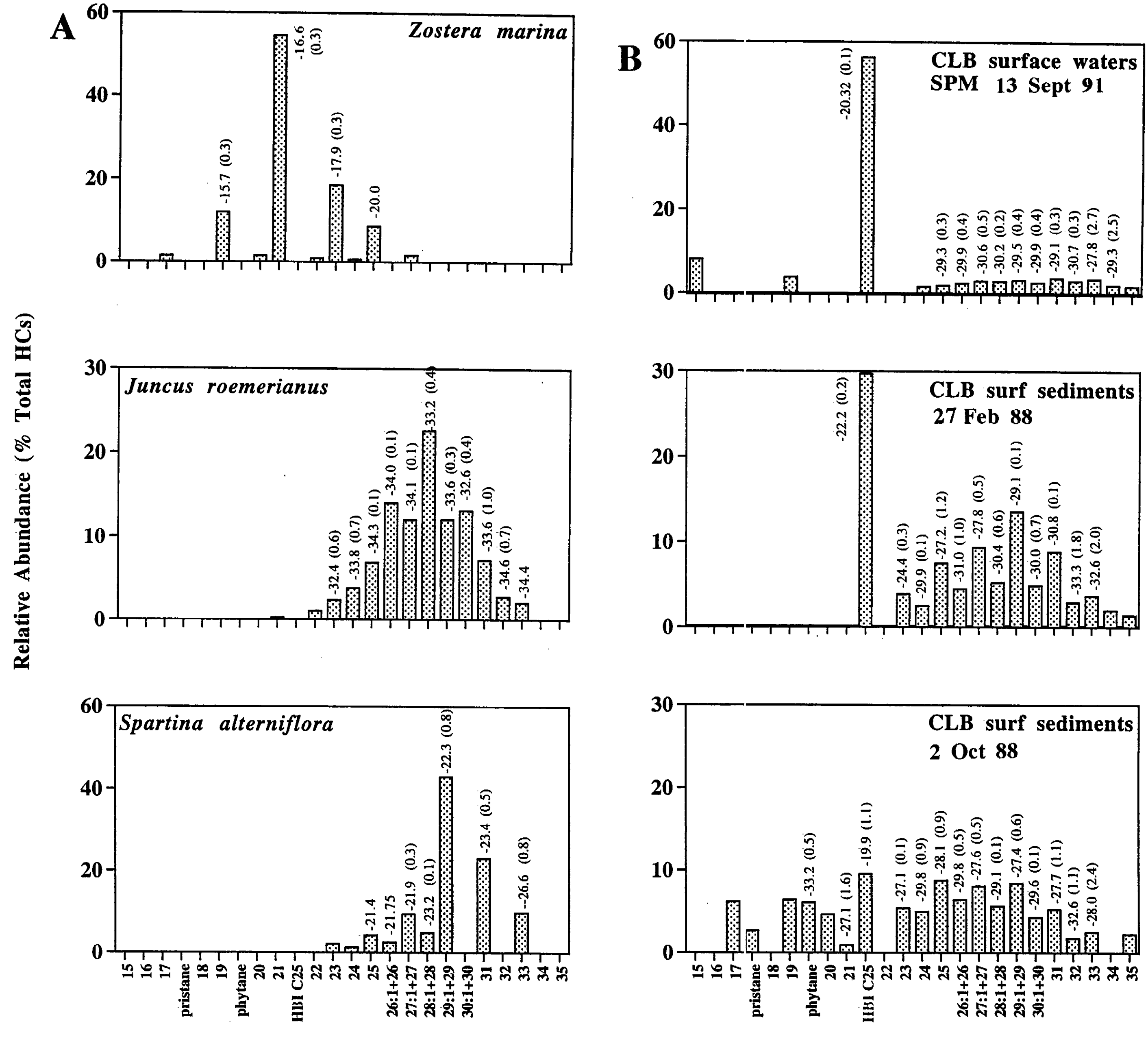

Fig. 4. Relative abundances of hydrocarbons (HCs, % total concentration) isolated from seagrass and marsh grasses (A), as well as suspended particulate matter (SPM) and surficial sediments (0–0.5 cm) collected from Cape Lookout Bight (CLB), North Carolina (B). Isotopic abundances ($\delta^{13}C$, per mil) for individual compounds (±SD) are listed above each bar. Total hydrocarbon concentrations ($\mu g\ g^{-1}$ dry wt sediment) were 26.8 and 13.0 for the February and October samples, respectively (Canuel and Martens 1993).

of inorganic carbon, isotope effects associated with carbon assimilation and metabolism, temperature effects, or a shift in the phytoplankton community structure to one with a greater abundance of ^{13}C-rich organisms (e.g. diatoms). Seasonally varying rates of phytoplankton productivity could also contribute to the isotopic variation. For example, the ^{13}C signature should increase due to a decline in $CO_2(aq)$ levels and(or) an increase in phytoplankton growth rates (Laws et al. 1995).

Previous studies have shown that rates of phytoplankton production in the shallow estuaries near CLB vary seasonally with maxima in late spring and summer (Thayer 1971). During periods of high algal growth rates, typically associated with high productivity, isotopic fractionation can decrease due to a rise in the rate of fixation relative to the supply of CO_2 across the membrane (Farquhar et al. 1982; Laws et al. 1995). This would suggest that isotopic enrichment is associated with high rates of productivity, which has been shown for other systems (Cifuentes et al. 1988). Likewise, temperature effects could also contribute to seasonal shifts in the isotopic composition of primary producers. Increases in temperature during summer would shift the equi-

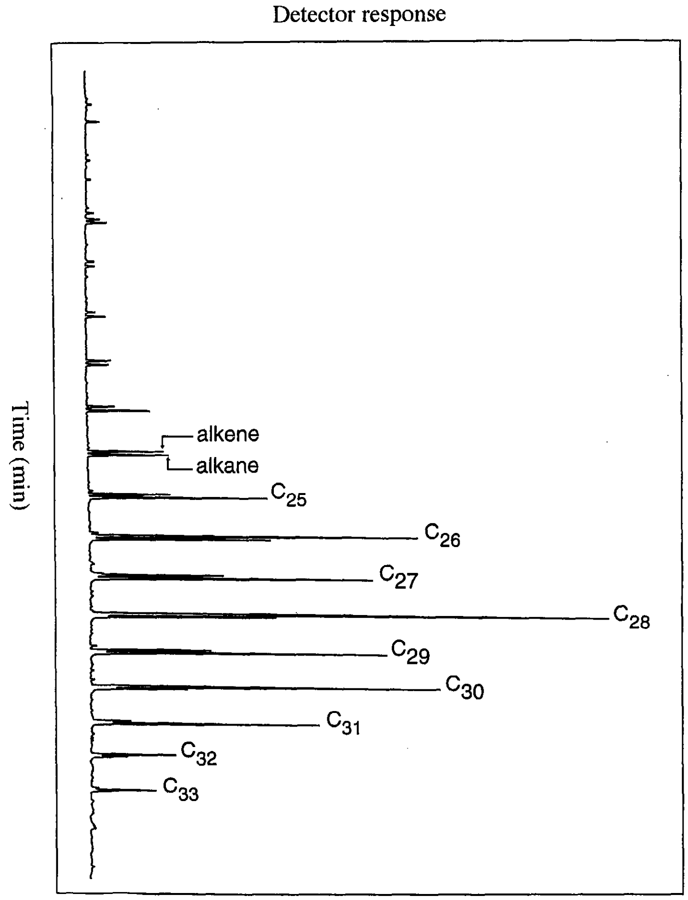

Fig. 5. Chromatogram showing abundance of *n*-alkenes and -alkanes in *J. roemerianus.* The abundance of the *n*-alkenes is sometimes equal to or greater than that for the *n*-alkanes (note that for each pair, the alkene elutes prior to the alkane). The *n*-alkane distributions are dominated by odd-numbered compounds whereas the alkene distribution is dominated by even-numbered homologues.

libria of inorganic carbon, lowering [CO_2(aq)] and leading to an enrichment in ^{13}C in CO_2(aq) (Mehrbach et al. 1973; Mook et al. 1974), although this effect should be small in the CLB system. A third possibility is that decreases in freshwater inputs to the nearby lagoons could also contribute to the seasonal shifts in isotopic abundance. The isotopic signature of riverine dissolved inorganic carbon (DIC) is typically lower than that for marine systems due to carbonate mineral dissolution in soils (Spiker 1980 and references therein). The isotopic composition of biomarker compounds generally attributed to phytoplankton (HBI C_{25}, 14:0, 16:1, and 24-methylcholesta-5,22-dien-3β-ol [28Δ5,22]) all showed enrichments in ^{13}C by 3–4‰ in the sediments collected in October vs. February, which is consistent with these possible mechanisms for isotope enrichment.

In addition to these metabolic and environmental factors, a change in phytoplankton assemblages to a ^{13}C-rich diatom-dominated community could contribute to the observed ^{13}C enrichment (Fry and Wainwright 1991). The biomarker distributions did allow us to consider whether increased inputs of diatom-derived organic matter could contribute to the shift in isotopic compositions observed during summer/fall. We find, however, that concentrations of diatom-derived lipids (e.g. 24-methylcholesta-5,22-en-3β-ol, 28Δ5,22) are actually higher in February than October (Canuel and Martens 1993), suggesting this is an unlikely explanation for the enrichments in ^{13}C observed in the sediments collected in October. Other studies in the region have shown that diatom blooms are characteristic of spring rather than summer or autumn (Litaker et al. 1993; Tester et al. 1995).

Alternatively, resuspended benthic microalgae from the shallow sounds surrounding CLB could be contributing to the isotopic shifts in $\delta^{13}C$ found in the CLB sediments. Low light levels and the absence of visible benthic microalgae (i.e. slimy brown filaments) in cores collected monthly over a 2-yr sampling period indicate that it is unlikely that benthic microalgae are living at the surface of CLB sediments. Information available for bulk carbon isotopes indicates that there is considerable variability in the isotopic signatures of benthic primary producers (-8.5 to -20.6; *see* Currin et al. 1995 for a summary). This variability may be due to poor sampling methods for separating the microalgal biomass from the sediment matrix, variations in productivity, and nutrient limitation in response to these fluctuations in productivity. Without information regarding the isotopic signatures for lipid biomarker compounds derived from benthic microalgae, we cannot exclude the possibility that benthic microalgae may contribute to the organic matter present in the sediments of CLB.

Isotopic signature of bacteria—An additional explanation for the seasonal shift in isotopic composition is that microbially mediated remineralization processes result in the removal of ^{13}C-depleted carbon and the subsequent accumulation of ^{13}C-enriched carbon during summer. To date, the effects of bacterial heterotrophy on the isotopic composition of lipids are not well understood. However, results from a study that examined the isotopic composition of bacterial biomass demonstrated that bacterial nucleic acids were enriched by up to 3‰ relative to their carbon source (Coffin et al. 1990). Eukaryotic heterotrophs are characteristically enriched in ^{13}C relative to their carbon substrate (DeNiro and Epstein 1978), and the magnitude of enrichment typically increases with trophic level. Such relationships have been used to elucidate pathways of carbon flow between substrates and consumers such as insects (Kenig et al. 1994), insects via mammals (Des Marais et al. 1980), and zooplankton (Logan et al. 1995). It is likely that the biomass of aerobic heterotrophic bacteria is also isotopically enriched relative to their carbon source, although further study of this requires a precise understanding of carbon substrate characteristics and dynamics, as well as carbon-use efficiency by the bacteria. Carbon assimilation by anaerobic bacteria is sufficiently different from that of aerobic heterotrophs that a simple expectation of similar enrichment between trophic levels would not be appropriate.

In the CLB surface sediments, isotopic enrichment could arise from seasonal changes in the substrate the bacteria use as a carbon source, variations in the microbial community structure, or associated metabolic pathways by which organic matter is remineralized. Results from a study by Martens et al. (1986) showed that the stable-carbon isotopic signature of biogenic methane varied seasonally and systematically in CLB sediments. Changes in the metabolic pathways by which methane production occurs and the cycling of key substrates such as hydrogen and acetate were proposed as potential mechanisms for fluctuations in the isotopic composition of sedimentary methane. Blair and Carter (1992) found that $\delta^{13}C$ values for porewater acetate, an important substrate for sulfate reducers and other microorganisms, varied from about -10 to -18 per mil in the surface sediments (upper 10 cm) of this site.

Of the compounds examined using irm-GCMS in this study, we find that bacterial fatty acids (e.g. 15:0, 17:1 + 17:0) were the most enriched in ^{13}C relative to other fatty acids. In sediments collected during October, bacterial fatty acids had isotopic abundances between -13 and -14‰. Isotopic enrichment of these compounds is also observed for sediments collected in February, when the isotopic signatures of these same compounds were -16 to -17‰. Furthermore, in comparing the isotopic composition of each of the fatty acid subgroups in the surface sediments (Table 2), we find that relative to other fatty acids, a greater isotopic enrichment occurs within the odd-numbered fatty acids, compounds generally thought to be derived from bacteria (Oliver and Colwell 1973; Parkes and Taylor 1983).

Based on results presented above and those of other investigators, lipids are generally depleted by 3–5‰ relative to $\delta^{13}C_{TOC}$. This suggests that the bacteria are using a carbon source with an isotopic signature of about -10‰. Although Blair and Carter (1992) found isotopically enriched acetate in pore waters collected from below 5 cm of CLB during summer months (July and August), acetate signatures in the surface sediments (<5 cm) were around -18‰. Thus, in the surficial sediments we examined, acetate is an unlikely source of isotopically heavy carbon. Alternatively, given the high levels of microbial metabolism in these sediments and the associated high demand for labile substrates, conditions approximating a closed system might result. As the same substrate is turned over subsequent times, it should become enriched in ^{13}C. Thus, in systems characterized by extensive recycling, one would expect the residual organic matter to become enriched in ^{13}C over time. While this might be the case for individual compounds, note that this effect is not observed in the downcore $\delta^{13}C_{TOC}$ values for CLB sediments (Blair and Carter 1992).

Incorporation of ^{13}C-enriched bacterial biomass into surficial sediments could provide a mechanism for the seasonal enrichments found in the surficial sediments of CLB during late summer/fall, although our results remain speculative. Without further investigation of the signatures that diagenetic processes leave on other, more abundant fractions of the sedimentary carbon, we cannot yet extrapolate from the ^{13}C enrichments we find in a few biomarker compounds to the processes responsible for the isotopic enrichments we found in the surficial sediments during summer and fall. Furthermore, our study has only examined the extractable lipid phase and it is possible that other sources, including vascular plants, may make important contributions to carbon sequestered in bound phases. Nonetheless, taken together, high rates of microbial activity, increases in the concentration of bacterial fatty acids, and the isotopic evidence presented here strongly suggest that microbially mediated processes contribute to the enrichments in ^{13}C found in the extractable CLB sedimentary organic matter. A diagenetic mechanism for the isotopic enrichments is also consistent with previous studies that have demonstrated that rates of carbon remineralization in CLB sediments are temperature dependent and generally three to four times higher during summer than at other times of the year (Crill and Martens 1987). In addition, recent evidence indicates that bacterial biomass increases in the surficial sediments during summer, a time when delivery of new sediment is low, suggesting that previously deposited organic matter is repackaged into bacterial biomass (Canuel and Martens 1993).

Recent investigations have also suggested the importance of bacterial biomass in surficial sediments (Kemp 1990) and its possible role in the long-term storage of sedimentary organic matter (Parkes et al. 1994). Although there is still considerable uncertainty surrounding the importance of bacterial biomass in the storage of sedimentary organic matter (Hartgers et al. 1994), our results indicate that further study in this area is warranted. Future studies should investigate the signatures that diagenetic processes leave on other, more abundant fractions of the organic matter present in sediments (e.g. bound lipids, kerogens, and pyrolysates). The CLB system with its high rates of carbon accumulation and remineralization would make an ideal field laboratory for investigating mechanisms for the incorporation of bacterial biomass into newly deposited sediments and its role in the long-term storage of sedimentary organic matter, with implications for the effects of these processes on sedimentary organic matter extending to other environments.

Conclusions

Lipids extracted from the seagrass *Z. marina* and the marsh grasses *S. alterniflora* and *J. roemerianus* displayed a range in molecular and isotopic composition. Consistent with the isotopic composition of bulk organic carbon ($\delta^{13}C_{TOC}$), lipid compounds extracted from *Z. marina* and *S. alterniflora* were enriched in ^{13}C relative to *J. roemerianus*. In comparison to bulk carbon, lipid biomarker compounds were, on average, depleted in ^{13}C by 3–5‰. Variations in the isotopic compositions of compounds isolated from these three classes of lipids, as well as differences between lipid compound classes, confirm that it is difficult to predict the source of organic matter to a sediment based on the bulk ($\delta^{13}C_{TOC}$) information alone. Further study of the composition of primary producers, as well as the biochemical and physiological factors controlling their isotopic composition, is necessary for reliable interpretation of biomarker compound distributions preserved in the sedimentary record.

A goal in determining the molecular and isotopic composition of these plants was to examine their importance as sources of sedimentary organic matter to coastal sediments. Despite high levels of productivity and high rates of export from surrounding marshes and seagrass beds, the results from this and previous studies indicate that seagrasses and marsh grasses make up a small fraction of the extractable sedimentary particulate organic matter accumulating in CLB. Future studies designed to resolve the fate of the vast amounts of carbon exported from the marshes and seagrass beds proximal to CLB should consider other approaches including an examination of bound lipids, other fractions of the sedimentary organic matter, or the use of other biomarkers.

Molecular and isotopic distributions suggest that phytoplankton derived from the overlying water column and sedimentary bacteria are the dominant sources of extractable organic matter accumulating in CLB. Although the biomarker signatures are similar in sediments collected during February and October, sediments collected in October were enriched in ^{13}C at the bulk ($\delta^{13}C_{TOC}$) and molecular level. Fatty acids derived from bacteria showed the greatest enrichment in ^{13}C, suggesting that the incorporation of isotopically enriched bacterial biomass may be an important process during late summer/fall.

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