BIOGEOCHEMICAL STUDY OF THE ALTAMAHA ESTUARINE SYSTEM: VARIATIONS IN ORGANIC MATTER INPUT AND BIOCHEMICAL REACTIVITY

by

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(Under the Direction of Ming-Yi Sun)

ABSTRACT

Estuarine systems receive organic matter from multiple sources (e.g. terrestrial vascular plants, salt marsh macrophytes, and marine/river phytoplankton), which is subsequently recycled through physical and biochemical processes. However, many questions remain unanswered. For example, how do organic matter inputs vary in different discharge seasons, how are the organic substrates from different sources utilized by estuarine organisms or degraded by related biochemical processes, and what are important factors controlling the bioreactivities of different organic material? My research aims to address these issues by combining field sample measurements and laboratory experiments. The analytical results indicate that chemical and isotopic compositions of the three typical end members in the Altamaha River estuary (marine diatom, salt marsh plant and land grass) changed differently from material to material and from compound to compound during degradation. There was a linkage (-3‰~-6‰ depletion) between $\delta^{13}C$ of bacteria-specific fatty acids and TOC, implying that $\delta^{13}C$ of bacteria-specific fatty acids can be used to trace the carbon sources of the microbial communities. The field observations indicate that terrestrial and marine sources dominated the organic matter inputs during the high discharge period while salt marsh plants contributed a large fraction at one site during the low

discharge period. In addition, organic matter deposited in the high discharge period was relatively fresh while that in low discharge period was highly degraded. The laboratory simulation experiments further demonstrate an order of biochemical reactivity for different materials: marine diatom > land grass > salt marsh plants. It appears that redox conditions had a small influence on degradation of fresh organic matter in aged sediments, while co-metabolism could have positive and negative effects on organic matter degradation, depending on the type of organic materials coexisting in the system and the diagenetic status of the materials. As primary catalyst in estuarine system, the bacteria communities preferentially utilized organic carbon from phytoplankton. But when salt marsh plants became a dominant input into the sediments at a given site during a low discharge period, bacteria efficiently used this organic matter as well.

INDEX WORDS: Estuary, Discharge, Chloropigments, Algal and terrestrial biomarkers, Bacteria-specific fatty acids, Lipid isotopic compositions, Lipid degradation, C4 Salt marsh plants, C3 marine diatom and land grass, Redox conditions, Co-metabolism, Labile and refractory organic matters, Principal component analysis, Multi-G model, Degradation rate constant.

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DEDICATION

I dedicate this dissertation to my husband for his unconditional support throughout and beyond this graduation work, and to my beautiful sons - my source of joy and energy that keep me moving forward. In addition, I have always received special love from my parents. It's truly impossible for me to express in words the level of gratitude I feel for everything they have done for me. Here I can just say: I love you very much, mom and dad!

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

Background

Estuarine systems act as a natural "sediment trap" to capture particulate and dissolved organic materials from both terrestrial and marine environments (Sholkovitz et al., 1978; Smith and Ellis, 1982; Kennedy, 1984; Canuel and Martens, 1993). They are physically complex zones in terms of turbulent mixing, settling/resuspension, organic matter burial and sedimentary mineralization, and thus play a disproportionately important role in the overall ocean carbon cycle (Pernetta and Milliman 1995; Gattuso et al. 1998). Rivers transport a tremendous load of organic carbon ($0.4x10^{15}$gC/year) into oceans, with approximately equal amounts of dissolved and particulate organic matter (Schlesinger and Melack, 1981). However, the current estimate of riverine organic matter input is primarily based on the studies of the 20 largest rivers in the world. The contributions of numerous smaller rivers have been ignored and thus leave a big uncertainty toward the global carbon cycling. Besides, although many studies have been successfully done with respect to biogeochemical processes in estuaries, it remains unclear how much organic matters from different sources is deposited in estuarine sediments and our understanding regarding the bioavailability of organic matter from different sources under variable environmental conditions is still incomplete (Hedges et al., 1988a, b; Kemp et al., 1996; Hopkinson et al., 1998; Boschker et al., 1999).

Marine and aquatic algae, salt marsh macrophytes, and terrestrial high plants are major sources of organic matter in estuaries (Wiegert et al., 1981, Mulholland and Olsen, 1992; Canuel and Martens, 1993; Shi et al., 2001). These end-member materials differ from each other with distinct chemical (e.g. C/N ratio and biomarker composition) and isotopic (e.g. stable carbon and nitrogen isotopic ratios) signatures, which and have been used to detect primary photosynthetic pathways, groups of organisms (e.g. phytoplankton vs. terrestrial vegetation), and food web relationships (Fry and Sherr 1984; Lajtha and Marshall, 1994; Fogel and Tuross, 1999). The isotopic signals of different organic materials are particularly useful in quantifying the relative contributions from each source to environmental organic pools based on the assumption that they behave conservatively without any significant change over the course of diagenesis (Peterson et al., 1985; Fogel et al., 1989; Eadie et al., 1994; Currin et al., 1995). However, a number of studies have indicated that substantial isotopic shifts could occur due to preferential decomposition of certain components within the total organic carbon pool, which was either isotopically enriched (i.e. carbohydrates and proteins) or depleted (i.e. lipids) (Spiker, & Hatcher, 1987; Benner, et al., 1987; Fischer, 1991; Hunkeler et al., 1999; Ahad et al., 2000). Because organic matter degradation is primarily microbially-mediated (Lee, 1992; Kemp, 1990; Parkes et al., 1994), the microbial processes may cause further isotopic changes to end-member substrates. However, the impacts are still contrasting from different studies (Wada et al., 1980; Harvey et al., 1995; Holmes et al., 1999). In spite of potential changes of isotopic signature in end-member materials during degradation processes, few studies have compared isotopic alterations between different organic materials and different compounds, which raises a big problem in precisely interpreting contributions of end-member materials in estuarine sediments.

Previous studies indicate that inputs from major sources of organic matter into estuarine

sediments varied greatly with location and time (Haddad and Martens, 1987; Canuel et al., 1997). For example, high concentration of algal organic matter usually follows a phytoplanktonic bloom (Harvey and Mannino, 2001) while highest terrestrial input occurs during high discharge period (Benner and Opsahl, 2001). Riverine discharge also strongly affects mixing of river water and seawater in the land-sea margin, which consequently causes a rapid resuspension/settling cycle and affects the quantity and quality of different organic matter entering the estuarine ecosystems. Previous research on the Altamaha estuary revealed the deposition pattern of particulate organic matter along the mixing zone during the high discharge season (Shi, et al., 2001). However, it is still unclear how the inputs and distributions of various organic matter varies in this estuary when the discharge becomes markedly low.

After organic material enters an estuary, it goes though dynamic degradation processes, depending on its bioavailability. It is generally believed that organic matter from algal sources is labile and readily decomposed while that from terrestrial sources is more refractory. However, accumulated evidence shows that the latter can be rapidly removed after entering the coastal system (Hedges et al., 1988a, b, 1997). Several factors are responsible for the variability in organic bioreactivity: (1) intrinsic structural features; (2) redox conditions; and (3) co-metabolism. Westrich and Berner (1984) indicated that organic matter had multiple fractions, which degraded at independent rates with their characteristic reactivities. Numerous recent studies further demonstrated that unsaturated bonds are more reactive than saturated bonds and short-chain lipids are more reactive than long-chain ones (Sun and Wakeham, 1994; Canuel and Martens, 1996; Harvey and Macko, 1997; Grossi et al., 2003). Diagenetic status (age) of materials were considered as another natural factor controlling bioreactivity. Canuel and Martens (1996) developed an approach for determining in situ decomposition rates of individual organic

compounds within parcels of sediments of known age. Their study demonstrated that organic matter reactivity changes with time, as fresh components degraded substantially faster than the aged ones. Conflicting conclusions have been reached so far regarding the effect of redox conditions on organic matter degradation from both observational and theoretical approaches (Henrichs and Reeburgh, 1987; Calvert and Pedersen, 1992; Canfield, 1989, 1993). Generally, oxic oxidation has been accepted as being more efficient in the degradation of organic matter than that of anoxic decomposition (Harvey and Macko, 1997; Sun et al, 1997, 2002; Teece et al., 1998; Hoefs et al., 2002). Other studies report that anoxic decomposition may not be intrinsically slower than oxic decomposition (Henrichs and Doyle, 1986; Lee, 1992). The reason why anoxic degradation is usually slower is because anaerobes often must deal with the "leftover" substrates (partially decomposed by aerobic bacteria) that tend to contain high proportions of refractory material (Wakeham and Canuel, 2006). The term "co-metabolism" describes a relationship between the degradation of refractory organic matter and labile organic matter, suggesting that high overall metabolic activity "fueled" by addition of labile organic matter can enhance the decomposition of more refractory components (Hughes and Parkin, 1991; Bianchi et al. 1997; Hedges et al., 1997). Canfield (1994) proposed a pseudo-G model to interpret this relationship. However, few experimental studies have been conducted to test this hypothesized model, especially at molecular level.

Hypotheses

Based on previous studies, four hypotheses are proposed to understand organic matter inputs and biogeochemical processes in the Altamaha River estuary:

(1) source signatures (chemical and isotopic composition) of organic inputs may be subject to significant diagenetic alterations in estuarine systems;

(2) organic matter inputs to the estuarine system may vary in different river discharge seasons due to the differences in organic matter delivery and mixing processes between high and low discharge periods;

(3) the microbial community may respond differently to organic material from different sources due to different metabolic adjustments on material quality (e.g. age and composition);

(4) biochemical reactivity of organic matter may change under variable environmental conditions (e.g. co-metabolism and redox conditions).

Methodology

Lipid biomarkers have been used as a powerful tool to examine organic carbon cycling in complicated estuarine systems due to the fact that they are source-specific compounds biosynthesized by specific organisms (Cranwell, 1982; Parsons et al., 1984; Canuel and Martens, 1993, Jeng et al., 1997). Generally, fatty acids consist of a major fraction of lipids. Among them, polyunsaturated fatty acids (PUFAs) such as 20:5, 20:4 and 22:6 and some monosaturated fatty acids (MUFAs) like 16:1(ω7) are thought to originate from planktonic materials (Canuel and Martens, 1993). For example, high value of 16:1(ω7)/16:0 ratio is usually an index of algal input (Volkman et al., 1989). Long-chain ($>C_{20}$) saturated fatty acids (LCFAs) are attributed to terrestrial vascular plants (Matsumoto et al., 1981). Branched iso- and anteiso-C_{13}, C_{15}, and C_{17} fatty acids, and 18:1 (ω7) are considered as exclusive indicators of bacterial sources (Volkman et al., 1980, Parkes and Taylor, 1983). Some short-chain saturated fatty acids (SCFAs) (C_{14}-C_{18}) and monounsaturated 18:1(ω9) have universal origins, including algae, bacteria, terrestrial and marsh plants (Cranwell, 1982). Neutral lipids also provide important information on carbon sources. For example, phytol, as an intermediate product of chlorophyll-a degradation, is often

used as an indicator of algal input (Hansen, 1980; Volkman and Maxwell, 1984; Sun et al., 1998). Long chain saturated fatty alcohols (C_{20}-C_{30}) are usually from terrigenous plants (Brassell et al., 1980) while short chain fatty alcohols (C_{14}-C_{18}) are generally from mixed origins. Sterols also carry signatures of different organic input although there are some uncertainties in their origins. Typically, $27\Delta^{5}$, $28\Delta^{5}$, $29\Delta^{5,22}$, $29\Delta^{5}$, $29\Delta^{5,24}$, $30\Delta^{5,22}$ are considered from mixed sources of zooplankton and algae, or higher plant and algae (Volkman, 1986) while $27\Delta^{5,22}$ and $28\Delta^{5,22}$ are mostly from marine plankton (Volkman 1986; Raven and Johnston 1991; Freeman et al., 1994, Canuel et al., 1997).

Although the biomarker approach has many advantages in tracing organic carbon sources, there are still several limitations in this approach due to: (1) the equivocal nature of some biomarkers from multiple sources (Gagosian et al., 1983; Volkman, 1986); (2) structural alteration by diagenetic processes (Hedges and Prahl, 1993); (3) incomplete information regarding the lipid composition in living organisms (Canuel and Martens, 1993, Summons et al., 1994); and (4) preferential disappearance of some source-specific lipids during transport and degradation. The simultaneous appearance of multiple lipid biomarkers and consistent source signatures in conjunction with other techniques (e.g. bulk parameter measurement and molecular isotope analysis) are thus required to elucidate the origins of various organic matter.

Stable isotopic analysis has enhanced the geochemical applications of biomarkers because of a broad range of carbon isotopic signatures as a function of carbon source and biosynthesis pathway (C_3 or C_4) (Freeman et al., 1990; Hayes et al., 1990; Rieley et al., 1991). Generally, terrestrial organic matter derived from C3 and C4 plants (including marsh plants) have distinct $\delta^{13}C$ ratios (-26‰ with C3 vs. -15‰ with C4 on average) while marine phytoplankton (C_3) has an intermediate $\delta^{13}C$ value (-20‰ on average) (Haines, 1976; Thayer et al., 1978; Fry and Sherr,

1984; Fry and Wainwright, 1991; Freeman et al., 1994). It is due to the distinctive isotopic signatures of different end-member substrates that bulk organic carbon isotopic value ($\delta^{13}C_{TOC}$) can be used to characterize the predominant sources of organic material in estuarine systems. The applications of bulk stable carbon isotopes in tracking sources of organic matter, however, have been limited by some uncertainties in the relative proportions of different organic inputs from various sources. For example, equal parts of C3 and C4 plant carbon combine to yield a TOM mixture with a $\delta^{13}C$ similar to C3 marine plankton and thus can lead to confusing compositional offsets in source assignments (Canuel et al., 1997).

Molecular $\delta^{13}C$ ratios of specific lipid biomarkers provide an additional tool to assess organic carbon sources since biomarkers from different carbon sources also have distinct molecular $\delta^{13}C$ ratios, although these are usually depleted by ~3-5‰ relative to bulk organic carbon pools (Hayes, 1993; Boschker et al., 1999). For example, 16:0 fatty acid is a universal compound derived from all organic carbon sources with different $\delta^{13}C$ ratios. During the degradation process of organic matter from various sources, changes in $\delta^{13}C$ ratio of 16:0 fatty acid can indicate which carbon source is preferentially degraded or preserved. On the other hand, the $\delta^{13}C$ ratios of bacteria-specific fatty acids may provide important clues regarding interactions between the bacterial community and organic sources. It was observed that when bacteria grew in systems with different substrates (with distinct $\delta^{13}C$), the same bacteria-specific fatty acids showed a clear difference in their isotopic ratios, parallel with the original difference in isotopic ratios of substrates (Boschker et a., 1999). However, bacteria grown in a system with a variety of carbon sources had $\delta^{13}C$ values relating to the predominant carbon distribution (Coffin et al., 1989). For example, the enrichment in ^{13}C may reflect the incorporation of isotopically heavier material, such as C4 plant, into bacterial biomass (Haines, 1976). It was also observed that a

facultative bacterium could biosynthesize the same bacterial fatty acids from the same substrate under oxic and anoxic conditions, but the fatty acids synthesized under anoxic conditions had a larger isotopic shift (~-8‰) from the original substrate isotopic ratio relative to those (~-2‰) synthesized under oxic conditions (Teece et al., 1999). Therefore, monitoring of bacteria-specific biomarker isotopic ratios would shed a light on what organic matter substrate (C3 vs. C4 or terrestrial vs. marine) is utilized by bacteria and which process (oxic vs. anoxic) is dominant.

Objectives

This dissertation focuses on three issues relating to biogeochemical carbon cycling in estuaries: (1) changes in chemical and isotopic signatures of organic material during degradation; (2) variability of organic matter inputs from different sources and bacterial responses during variable discharge periods; (3) bioreactivity of different organic materials under various environmental conditions. The specific objectives are:

(1) to examine the effects of organic matter degradation on the chemical and isotopic compositions of three typical plant materials in the Altamaha estuary (land grass, salt marsh plant, and marine diatom);

(2) to determine the spatial distributions of organic matter by measuring bulk C/N parameters, chlorophyll, and lipids in surface sediments along the land-sea margin in the Altamaha River during both high and low discharge seasons;

(3) to differentiate the relative contributions of organic matter from different sources by combining end-member model, PCA and biomarker approaches;

(4) to determine the degradation rate constants of organic components by following variations of lipid biomarkers in sediment and water incubations with addition of end-member organic

materials (land grass, salt marsh plant, and cultured marine diatom);

(5) to assess the factors affecting the degradation processes of organic matter, including the nature of the substrate (e.g. structural features and age), redox conditions, and co-metabolism;

(6) to evaluate the bacterial response to variable organic matter inputs by measuring isotopic compositions of bacteria-specific biomarkers in field and incubation samples.

Organization of this dissertation

This dissertation consists of six chapters: an introduction, four main chapters and a summary. Each chapter is independent in format, but inherently linked, as they are all focused on biogeochemical studies of organic matter in the Altamaha estuarine system.

Chapter 2 reports on a two-month incubation experiment simulating aerobic degradation of three typical end-member materials in Altamaha estuarine water: C3 land grass (*Festuca arundinacea*), C4 salt marsh plant (*Spartina alterniflora*), and C3 marine diatom (*Skeletonema costatum*). The purpose of this study is to characterize the effects of early diagenesis on chemical and isotopic signatures of different organic materials, which is critical in assessment of input and transport of organic carbon in estuaries. Variations of bulk parameters (including TOC, TON, C/N ratio, $\delta^{13}C$, and $\delta^{15}N$) and multiple lipid classes (fatty acids, neutrals and sterols) were followed over the course of the incubation. Special effort was made to compare the $\delta^{13}C$ between bacteria-specific fatty acids and bulk materials in order to discuss the linkage between microbes and bioavailable substrates.

Based on the findings in Chapter 2, Chapter 3 investigated the spatial and temporal distributions of organic matter from variable sources in the Altamaha River during high and low discharge periods. In addition to the chemical and isotopic analyses at bulk and molecular levels,

three-end-member modeling was constructed to quantify the relative contributions of C3 terrestrial and marine plants and C4 salt marsh plants at each sampling station. Principal component analysis (PCA) was used to infer two major factors in controlling the organic matter distributions in this land-sea margin: diagenesis status (fresh vs. degraded) and source (autochthonous vs. allochthonous). The study further evaluated bacterial responses to changes in organic inputs by following the isotopic ratios of bacteria-specific fatty acids and their compositional relationship with Chl-a and other lipid subgroups.

Chapter 4 describes a time-series incubation designed to test how redox conditions and co-metabolism affect the biochemical reactivities of organic material from various sources in estuarine sediments. The three typical substrate materials of the Altamaha River (marine diatom *Skeletonema costatum*, land grass *Festuca arundinacea* and salt marsh plant *Spartina alterniflora*) were added to pre-incubated (aged) sediments either separately or together. Emphasis was placed on a comparison of degradation rate constants of TOC and major fatty acids in different material treatments. Variation patterns of bacteria-specific biomarkers and their stable carbon isotopic ratios were tracked in order to evaluate the uses of organic matter by bacterial communities in the different treatments.

Chapter 5 followed the plan of Chapter 4 but focused on examining the effect of co-metabolism on degradability (bioreactivity) of organic matter in estuarine water. In contrast to chapter 4 where single fresh substrates were utilized, the three typical organic materials (marine diatom, land grass and salt marsh plant) with different diagenetic status were deliberately combined to represent variable mixtures of fresh vs. aged and labile vs. refractory materials. The incubation was carried out in oxygenated seawater for one month and a two-component degradation model was applied to determine the degradation rate constants of fatty acids. The

discussion was focused on: (1) if the intrinsic factors, including diagenetic status (age) and molecular structure, affect the bioreactivity of organic matter; and (2) if the addition of labile organic materials enhances degradation of refractory organic matter. As key catalytic agents in organic matter degradation, bacterial communities were examined by following the chemical and isotopic signatures of bacteria-specific fatty acids.

Chapter 6 summarizes the findings in the four main chapters, stressing the implication of this study with respect to organic input, bioreactivity and the corresponding bacterial response in estuarine systems.

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CHAPTER 2

CHANGES IN CHEMICAL AND ISOTOPIC SIGNATURES OF PLANT MATERIALS DURING DEGRADATION: IMPLICATION FOR ASSESSING VARIOUS ORGANIC INPUTS IN ESTUARINE SYSTEMS

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Abstract

To evaluate the applicability of the end-member mixing model in assessment of input and transport of organic carbon in estuarine system, we incubated marine diatom, land grass, and salt marsh plant in Altamaha estuarine water for two months. Chemical and isotopic parameters (bulk organic carbon/nitrogen contents, lipid compositions, stable C/N isotopes, and lipid stable carbon isotopic ratios) were analyzed for fresh and degraded materials. The results showed that although the C/N and $\delta^{15}N$ ratios of three materials varied similarly during degradation, the bulk $\delta^{13}C$, lipid compositions, and lipid stable carbon isotopic compositions varied differently from material to material and from compound to compound, implying that applications of end-member model should consider the diagenetic status of organic materials and the potential changes in chemical and isotopic signatures.

Keywords: Organic matter inputs, Plant materials, Chemical and isotope signatures, Lipid composition, Degradation, End-member model, Bacteria-specific fatty acids.

Introduction

Organic carbon flow crossing the land-sea margin is an important term in global carbon cycle and its estimate depends on identification of carbon sources and end-member modeling (Hedges et al., 1997). Aquatic algae, salt marsh macrophytes, and terrestrial high plants, with distinct chemical and isotopic signatures, are major sources of organic matter in estuarine system (Wiegert et al., 1981) and have been used as end-member materials to study carbon cycling in estuarine and coastal environments (Fry and Sherr 1984). Applications of the end-member model are based on a fundamental assumption that the isotopic signatures of original materials are not significantly altered by degradation processes (Peterson et al., 1985).

Many studies have observed isotopic alterations of organic matter in natural environments, which are attributed to preferential decomposition of labile components over refractory components (Spiker and Hatcher, 1987). Some organic compounds experience isotopic fractionation during degradation (Meckenstock et al., 1999) while other organic compounds resist isotopic alteration (Hayes et al., 1990). Sun et al. (2004) observed that the isotopic signatures of different lipid biomarkers (compound specific carbon isotope ratios) in a single phytoplankton were altered differently (enrichment, depletion, and no change) during degradation. To date, few studies have compared isotopic alterations between different organic materials and different compounds during degradation.

This study examined the effects of organic matter degradation on the chemical and isotopic signatures of marine diatom, salt marsh plant and land grass. Three fresh organic materials were incubated in Altamaha estuarine water for two months. The chemical and isotopic compositions for fresh and degraded materials, including bulk organic carbon/nitrogen contents, stable C/N isotopes, lipid compositions and their stable carbon isotopic ratios were determined. Isotopic

compositions of bacteria-specific fatty acids were also measured to examine the potential link between microbes and bioavailable substrates.

Experimental

The C3 marine diatom *Skeletonema costatum* (clone CCMP1332) was obtained from the center for Culture of Marine Phytoplankton, Booth Bay Harbor, ME, USA. It was cultured in F/2 medium with a 12:12 light/dark cycle at 16ºC. The algal cells were harvested and stored frozen (-40ºC) for later incubation. The C4 salt marsh plant *Spartina alterniflora* was collected from Sapleo Island, Georgia, USA. The C3 land grass *Festuca arundinacea* was collected in the upland of Georgia. Fresh leaves were cut from plants and stored frozen (-40ºC) prior to the experiment. The seawater (salinity ~28‰) was collected at the Altamaha River mouth and filtered with a glass fiber filter (0.5 μm).

Plant leaves were cut into small pieces (~2 × 2 mm) and ~10 g of each material was separately added into three flasks with ~2L seawater. The incubation was carried out in a 12:12 light/dark regime at 16ºC with flasks shaken twice a day. The water was continuously purged with air to keep aerobic conditions. Detritus were collected by centrifugation at the end of experiment. A small aliquot (~1 g) of each wet material was used for chemical and isotopic analyses.

Total organic carbon (TOC) and nitrogen (TN), stable carbon/nitrogen isotopes ($\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$) of fresh plants and detritus were determined using a Carlo Erba CHN analyzer coupled with a Finnigan Delta C Stable Isotope Mass Spectrometer. Lipid extraction and separation were conducted based on the published procedure (Sun et al., 1997). Fatty acids and neutral lipids were analyzed with a Hewlett-Packard 6890 GC. The lipid compounds were

identified with a Shimadzu QP-5000 GC-MS system. Stable isotopic ratios of individual lipids were determined using a GC-combustion system linked to a Finnigan MAT 252 isotope ratio mass spectrometer. The $\delta^{13}C$ values of individual compounds were calculated based on the SFC CO_2 (99.999%) reference gas ($\delta^{13}C$ = -11.23‰ relative to PDB) and corrected for addition of extra carbon atoms during their derivatization.

Results and discussion

Changes in bulk parameters. Each of the three fresh materials possessed distinct bulk chemical and isotopic signatures (Table 1). The C/N ratio of diatom was the lowest while that of salt marsh plants the highest. The $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ of three materials are in a typical range of natural C3 and C4 plants. After incubation, TOC and TN contents of three materials decreased differently: 63% of TOC and 57% of TN lost in diatom; 44% of TOC and 35% of TN lost in land grass; and only 13% of TOC and 12% of TN lost in salt marsh plant. Meanwhile, their C/N ratios generally decreased by 1-2. Although diatom and land grass lost more carbon than salt marsh plant during incubation, their $\delta^{13}C_{TOC}$ ratios had less changes (+0.69‰ and -0.17‰) than that of salt marsh plant (+1.99‰). On the other hand, the bulk $\delta^{15}N$ ratios of three materials increased by +3‰ to +7‰.

Marine phytoplankton predominately consists of labile proteins, carbohydrates, and lipids (Parsons et al., 1961) while terrestrial organic matter contains a large fraction of refractory components such as hemicellulose, cellulose, and lignins (Opsahl and Benner, 1995), resulting in faster degradation of marine organic matter than terrestrial organic matter. The relative decreases in TOC and TN of three materials after incubation were consistent with the reactivity pattern of different organic materials. However, during incubation, bacteria biosynthesized new biomass

and affect the bulk parameters. For example, the C/N ratios in all three degraded materials decreased, while in natural environments C/N ratios usually increase during degradation due to faster decomposition of labile nitrogen-containing compounds. The major reason for the inconsistency could be attributed to a significant production of bacterial biomass in our experimental systems. Although nitrogen in plant materials is preferentially removed, newly produced bacterial biomass can cause a decrease in the total C/N ratio since the C/N ratio of bacterial biomass is generally low (Müller, 1977). Harvey et al. (1995) observed that bacterial carbon reached to ~10% of total carbon at a given stage of phytoplankton degradation.

Apparent enrichments (+3‰ to +7‰) in $\delta^{15}N$ of three materials are likely caused by preferentially loss of isotopically lighter components or gain from newly produced isotopically heavier compounds. Sachs and Repeta (1999) found that $\delta^{15}N$ ratios of suspended particles increase with depth in water column due to the leaching of ^{15}N-depleted nitrogen during decomposition. Bacteria produce ^{15}N-enriched (~+3‰) proteins (Macko et al., 1987) relative to the bulk nitrogen while protein hydrolysis yields further ^{15}N-enrichment (Bada et al., 1989). Little (-0.17‰) or relatively smaller variations (<+2‰) in $\delta^{13}C_{TOC}$ of three degraded materials may indicate a net balance of counteracting processes of plant organic matter degradation and bacterial biosynthesis. Protein in plant materials is enriched in $\delta^{13}C$ and more labile compared to celluloses, lignins, and lipids (Degens et al., 1968). Preferential loss of the labile but isotopically heavier components results in ^{13}C depletion of remaining organic detritus (Benner et al., 1987; Lehmann et al., 2002). However, bacterial biomass is usually enriched in ^{13}C relative to the substrate (Macko and Estep, 1984). Therefore, when the influences of two opposite processes are canceled out, the variations in ^{13}C of total carbon will be minimized.

Changes in lipid compositions. Remarkable changes in fatty acid compositions were observed for three plant materials after incubation (Fig. 2.1a). Fresh diatoms contained several polyunsaturated fatty acids (e.g., 20:4+20:5, 22:6) as significant components while fresh land grass and salt marsh plant were dominated by 18:2+18:3 polyunsaturated fatty acids. There were almost equal amounts of monounsaturated 16:1 and saturated 16:0 fatty acids as major components in the fresh diatoms. After incubation, polyunsaturated and monounsaturated fatty acids in three materials dramatically declined (60-95% for 16:1, 18:1(ω9), and 18:2+18:3) and even completely disappeared (for 20:4+20:5 and 22:6) while saturated fatty acids (16:0 and 18:0) became dominant components (50-75%) in the detritus. Some bacteria-specific fatty acids (branched 15:0 and 17:0, and 18:1(ω7)) were noticeably produced during incubation. Fewer variations occurred for neutral lipid compositions of three materials (Fig. 2.1b). The most abundant neutral lipid compound in diatom and land grass was phytol, while in salt marsh plant, phytol and a C_{29} sterol (24-ethylcholest-5-en-3β-ol, ($29\Delta^5$)) were almost equally abundant. There were also several C_{27}-C_{29} sterols present in the plant materials. Small amounts of short-chain (C_{14} and C_{16}) alcohols (C_{18} was an exception) were found in fresh diatoms but only a little C_{12} alcohol was present in fresh land grass and salt marsh plant. After incubation, the relative abundances of major neutral lipids (phytol and $29\Delta^5$ sterol) varied less than 12% in all detritus.

A major factor responsible for the large changes in fatty acid composition during degradation was the structural feature (unsaturation vs. saturation). Many field observations and laboratory experiments have shown that unsaturated, especially polyunsaturated, fatty acids are preferentially degraded than saturated fatty acids (Haddad et al., 1992; Sun et al., 1997). The relative ratios of some unsaturated to saturated fatty acids (e.g., 16:1/16:0) have been used as indicators for organic matter inputs from algal bloom (Volkman et al., 1989). Our experimental

results suggest that the variations in relative unsaturated/saturated ratios are closely related to the degradation extent. Thus, they can serve as a useful index for diagenetic status of various organic materials in natural environments. For example, very low 18:2+18:3/16:0 ratios in samples may imply considerable degradation of land and salt marsh plants while a 16:1/16:0 ratio close to 1 may indicate a large input from fresh phytoplankton.

Relatively constant compositions of major neutral lipids after degradation may be due to the similarity in degradation rates although their structures are very different. Sterols are generally thought to be recalcitrant components in the lipid pool but some evidence showed that they degraded at comparable rates to fatty acids and phytol under oxic conditions (Harvey and Macko, 1997). Therefore, degradation of organic materials caused marked losses of these neutral lipids but did not largely alter their relative abundances. This consistency provides an advantage for application of end-member modeling in natural environment because it is unnecessary to distinguish these biomarkers between fresh and degraded materials.

Alterations of lipid isotopic compositions. Four lipid compounds (14:0 and 16:0 fatty acids, phytol, and $29\Delta^5$ sterol) were chosen for examination of variations in $\delta^{13}C$ because they were present in all fresh and degraded materials at detectable levels. Through degradation, the molecular $\delta^{13}C$ ratios varied from material to material and from compound to compound (Fig. 2.2a). In most cases, surviving compounds became enriched in ^{13}C (up to +8.26‰) with only one exception for significant depletion (-2.6‰ for 16:0 in diatom). Larger shifts in molecular $\delta^{13}C$ ratio after degradation were observed for land grass than for diatom and salt marsh plant when compared with the compounds. However, if compared with the materials, $\delta^{13}C$ ratios of fatty acids seemed to be altered at a larger scale than those of neutral lipids. The shifts for neutral lipids in most cases were less than 1‰. Even though most compounds became enriched in ^{13}C

after degradation, they were still isotopically lighter than the bulk carbon of materials.

As indicated by these results, degradation processes significantly affect the isotopic signatures of organic matter. However, the influences varied from material to material and from compound to compound. In land grass, the largest changes in lipid $\delta^{13}C$ after degradation were accompanied with the least shift in bulk ^{13}C, while in salt marsh plant the largest bulk $\delta^{13}C$ shift was coincident with the least changes in lipid $\delta^{13}C$. Inverse variations of isotopic shifts between total carbon and lipids imply that a small change in $\delta^{13}C_{TOC}$ does not mean absence of isotopic fractionation for all organic compounds.

There are two possible causes responsible for the difference in isotopic shift between fatty acids and neutral lipids. First, bacterial growth during incubations produces new fatty acids in their biomass but no phytol and $29\Delta^5$ sterol were synthesized. Contribution of bacterial fatty acids, with distinct $\delta^{13}C$ from plant fatty acids, may alter the fatty acid molecular isotopic compositions (Canuel et al., 1997). Second, degradation pathways of fatty acids and neutral lipids are very different. Fatty acids are degraded mainly through decarboxylation (Sun et al., 1997) while the carbon at carboxyl group of fatty acids is isotopically lighter than overall carbons bound in the chain (deNiro and Epstein, 1997). Thus, the degradation of fatty acids results in enrichment in ^{13}C for surviving compounds. In contrast, degradation of sterols is primarily through interconversion pathways (producing stenones, stanones, stanols, steroid diols, and sterenes), which do not involved carbon number changes (Gagosian et al., 1980). Therefore, isotopic fractionation for sterols during degradation may be insignificant, which was confirmed by other studies (Sun et al., 2004).

Link between substrate materials and bacteria-specific fatty acid $\delta^{13}C$ ratios. In three separate incubation systems, bacterial growth was dependent on different organic materials,

which had distinct bulk $\delta^{13}C$ ratios. During incubation, bacteria produced some specific compounds such as iso-15:0, anteiso-15:0, and 18:1(ω7) fatty acids and their molecular $\delta^{13}C$ ratios seemed to be related to the isotopic ratios of bulk organic materials (Fig. 2.2b). Like the bulk carbon pools, there were distinguishable differences in the $\delta^{13}C$ of bacteria-specific fatty acids among three incubation systems.

A laboratory experiment (Teece et al., 1999) demonstrated that aerobic and anaerobic bacteria used different pathways to synthesize bacteria-specific fatty acids, resulting in distinct isotopic compositions for these biomarkers. Aerobic bacteria biosynthesize fatty acids using acetyl-CoA produced by pyruvate dehydrogenase enzyme, with a similar $\delta^{13}C$ to that of substrate. In contrast, anaerobic bacteria produce fatty acids using formate via the serine pathway and these compounds are much depleted (up to -12‰) in ^{13}C relative to the carbon source. In our experimental systems, seawater is oxygenated and aerobic bacteria dominate in the community. So, the bacteria-specific fatty acids carried the stable carbon isotopic compositions similar to those of substrate materials. Therefore, the similarity in $\delta^{13}C$ ratios between bacteria-specific fatty acids and substrate materials provides an insight to the link between microbes and bioavailable substrates in estuarine systems.

Conclusions

Experimental results showed that degradation of organic materials not only changed their chemical compositions but also altered the isotopic signatures of total carbon and individual compounds. Therefore, the assumption of end-member modeling needs to be reassessed based on the freshness and type of organic materials, and potential alterations in chemical and isotopic compositions. In summery, understanding for the effects of degradation on chemical and isotopic

compositions of organic materials in estuarine systems may refine our previous view for carbon flow between land and ocean.

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Table 2.1. Bulk carbon and nitrogen contents, C/N ratios, and C/N isotopes of fresh and degraded plant materials.

	Marine diatom		Land grass		Salt marsh plant	
	Fresh	Degraded	Fresh	Degraded	Fresh	Degraded
TOC (%)	19.1	6.9	39.1	22.0	40.5	35.4
TN (%)	2.7	1.2	2.8	1.8	1.4	1.2
C/N (mole/mole)	8.2	6.9	16.5	14.2	34.0	33.5
$\delta^{13}C_{TOC}$ (‰)	-20.16	-19.47	-29.79	-29.96	-13.20	-11.21
$\delta^{15}N_{TN}$ (‰)	-1.40	1.70	0.39	7.58	6.64	10.18

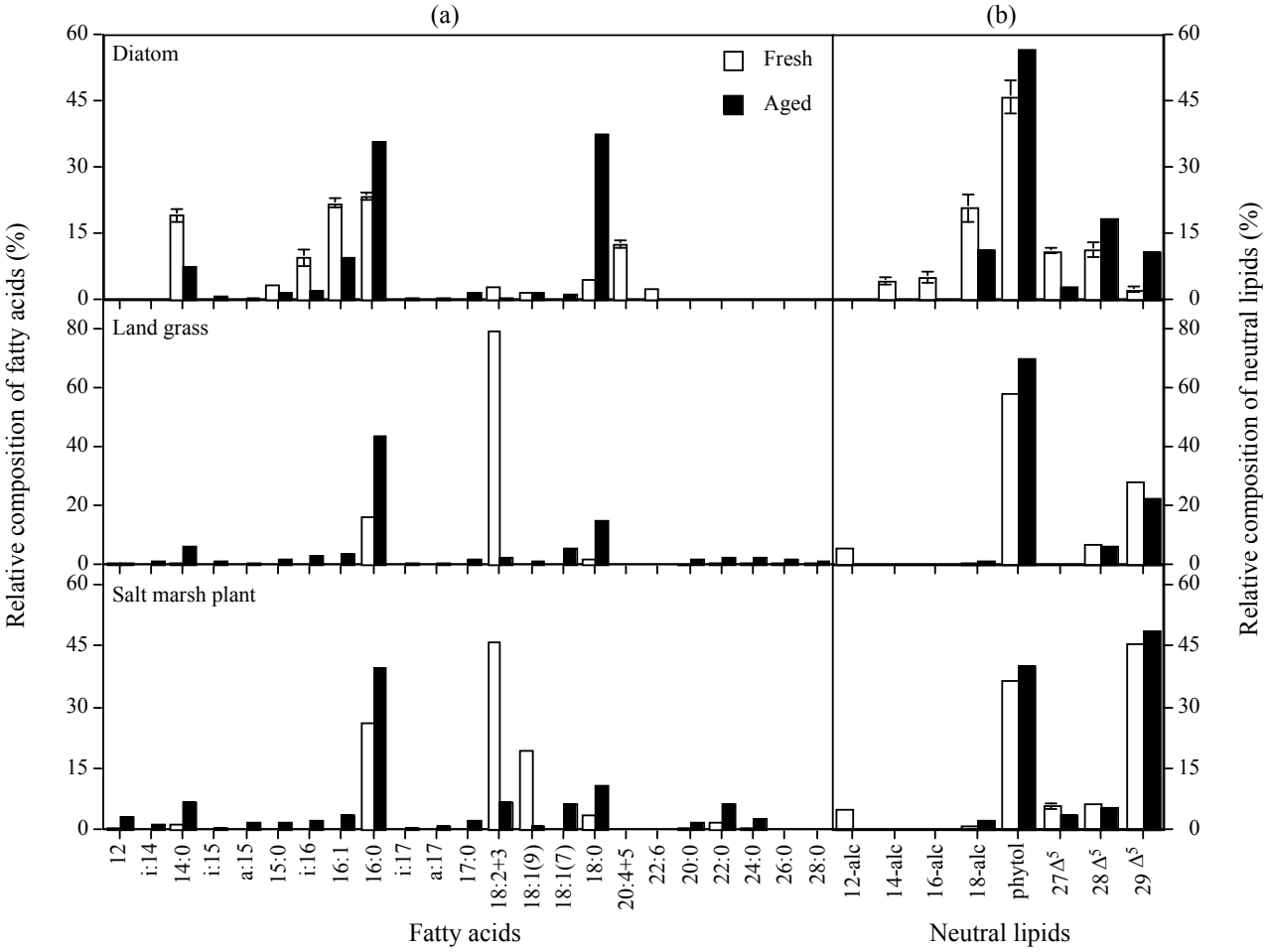

Fig. 2.1. Relative lipid compositions of the fresh and degraded organic materials (diatom, land grass, and salt marsh plant): (a) fatty acid compositions and (b) neutral lipid compositions.

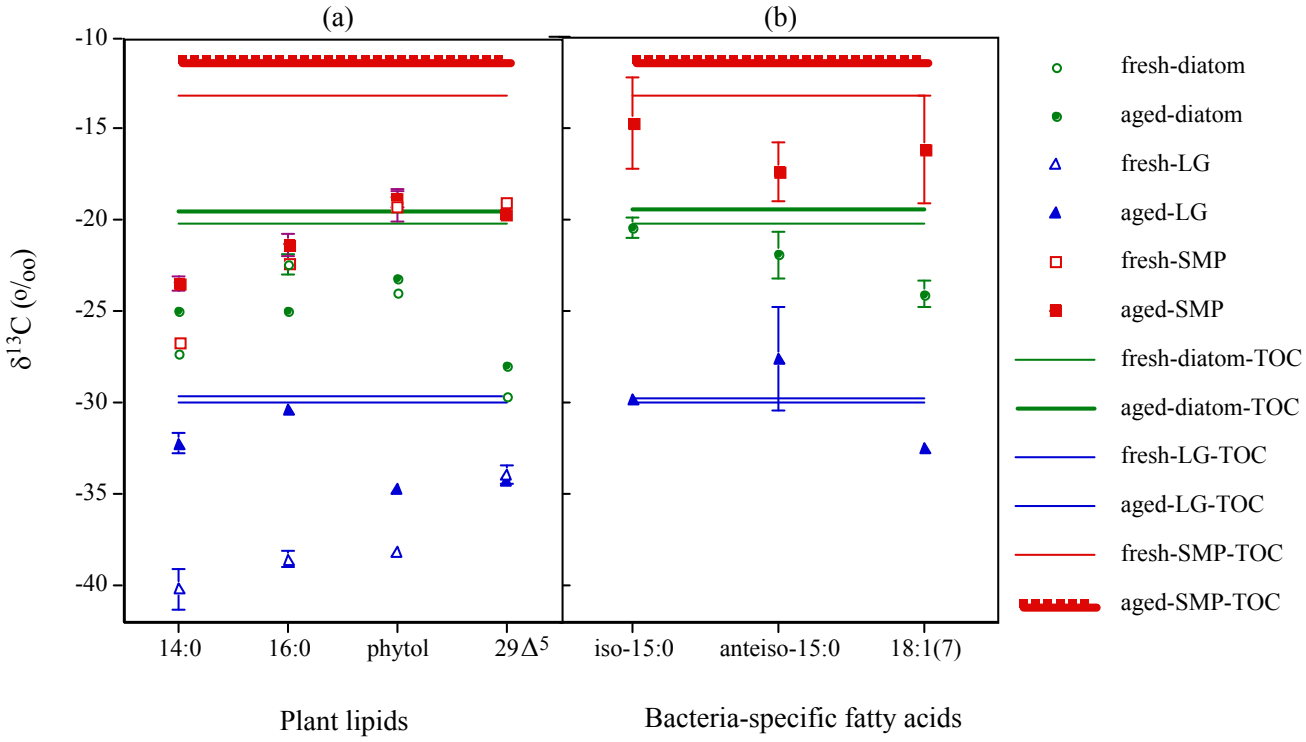

Fig. 2.2. Variations of stable carbon isotope ratios of lipids derived from fresh and degraded plant materials: (a) plant lipids and (b) bacteria-specific fatty acids.

CHAPTER 3

ORGANIC MATTER SOURCES AND THEIR USE BY BACTERIA IN THE SEDIMENTS OF THE ALTAMAHA ESTUARY DURING HIGH AND LOW DISCHARGE PERIODS

[1]Dai, J. and M.-Y. Sun. Accepted by Organic Geochemistry, 10/16/2006.

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Abstract

To understand the influence of river discharges on the biogeochemical cycling of organic matter (OM) in estuarine sediments, we conducted chemical and isotopic analyses of surface sediment samples collected from the Altamaha estuary (Georgia, USA) in March 2002 (high discharge period) and October 2002 (low discharge period). Chemical and isotopic analyses included bulk parameters (TOC, TN, $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$), chlorophyll-a (Chl-a), lipids (fatty acids, alcohols, and sterols) and lipid carbon isotopic compositions. Bulk parameters were apportioned with a three-end-member mixing model and biomarkers were processed using the principal component analysis (PCA). The modeling indicated that OM from C3 terrestrial and marine sources dominate (>80%) at most sediment sites during the two periods while C4 salt marsh plants contribute a large fraction (~40%) of OM at one site during the low discharge period. The PCA demonstrated that more allochthonous OM deposits in the high discharge period while more autochthonous OM accumulates in the low discharge period; OM in the high discharge period is relatively fresh while that in the low discharge period is highly degraded. Distributions of bacteria-specific fatty acids in the estuarine sediments followed the same patterns as Chl-a, algal lipids, and mixed lipids but were not correlated with terrestrial lipids, suggesting that the microbial community in the sediments depends largely on OM from phytoplankton rather than from terrestrial higher plants. When salt marsh plants became an important input into the sediments, bacteria efficiently used this OM, indicated by the coincident changes between molecular isotope ratios of bacteria-specific fatty acids and bulk $\delta^{13}C_{TOC}$.

Keywords: Altamaha estuarine sediments, River discharge, Organic matter sources, Biomarker distributions, Isotope compositions, Bacterial response, End-member modeling, PCA approach

Introduction

Estuaries are important traps for materials from both land and ocean (Bopp et al., 1982). Flocculation and coagulation during mixing of freshwater and seawater results in a rapid deposition of materials to the sediments. The organic matter in estuarine sediments originates from many sources, including terrestrial higher plants, marine phytoplankton and zooplankton, benthic animals, macroalgae and marsh plants (Hopkinson, 1985). Input of these different organic materials varies with the plant life cycle, production patterns, transport pathway, and environmental conditions (Wiegert et al., 1981, Harvey and Mannino, 2001). Changes in river discharge over time also affect the biogeochemical cycling of organic matter in estuarine systems (Benner and Opsahl, 2001). However, few studies have addressed the influence of river discharge on organic matter inputs into estuarine sediments (Otero et al., 2000). Moreover, it has been unclear how sediment microbial communities respond to such variations of organic inputs (Bouillon and Boschker, 2006).

The Altamaha River is an important drainage basin on the Georgia coast, with a remarkable seasonal variation in discharge. Otero et al. (2000) reported that microalgae-derived material was the dominant component of POM in the river water during summer (a period of high aquatic productivity) while land plant-derived organic matter was more important during spring (a high discharge period). Shi et al. (2001) found that terrestrial OM and marine-originated OM was deposited at different mixing sites in the Altamaha estuary during the high discharge season. However, it was not known how distributions of various organic matter might vary in Altamaha estuarine sediments when the river discharge is low.

When organic materials deposit into estuarine sediments, they are utilized by benthic communities (Hedges et al., 1988). Microbial processes appear to be the principal mechanism for

organic matter degradation (Kemp, 1990), although animals also have a role. Some studies have demonstrated that marine-derived organic matter is more readily degraded in estuarine systems than those from the land (Ittekkot and Lanne, 1991; Canuel and Martens, 1996). However, other studies have argued that metabolic consumptions of both autochthonous and allochthonous organic matter are almost equally important in many estuarine systems (Hopkinson and Vallino, 1995; Smith and Hollibaugh, 1995). More evidence (Hedges et al., 1997) has showed that terrestrial organic matter is rapidly recycled in estuaries and coastal oceans, but it remains unclear how the refractory land-derived organic matter is degraded in these systems.

Organic materials from different sources in estuarine sediments have distinct chemical and isotopic compositions. Generally, the organic matter in terrestrial higher plants synthesized via the C3 pathway is more depleted in ^{13}C (-31‰ to -26‰) than in C4 salt marsh plants (-16‰ to -12‰) while C3 phytoplankton has an intermediate $\delta^{13}C$ values (~-20‰ on average) (Fry and Sherr, 1984; Stribling and Cornwell, 1997). Moreover, terrestrial and salt marsh plants, which contain high content of nitrogen-free macromolecules such as lignin, tannin, hemicellulose, cellulose, cutin, and suberin, generally have higher C/N ratios (20–500) compared to marine plankton (~7) (Hedges et al., 1997). A combined lipid biomarker and molecular stable carbon isotope approach has been applied to assess the sources of organic matter in natural environments (Hayes et al., 1990; Boschker et al., 2005). When a large and complex data set is involved, multivariate statistic analyses (e.g., principal component analysis, PCA) have been demonstrated to be useful for tracking patterns of organic matter distributions (Yunker et al., 1995; Zimmerman and Canuel, 2001).

This study has two specific goals: (1) to examine the variations of organic matter sources in Altamaha estuarine sediments during high and low discharge periods; and (2) to determine

microbial response to variable organic inputs. We conducted chemical and isotopic analyses for surface sediment samples collected from nine sites along the salinity gradient in the Altamaha estuary in March 2002 (high discharge period) and October 2002 (low discharge period). Data were treated with a three-end member model and the PCA approach.

Experimental

Study area and sampling sites. The Altamaha River is the third largest contributor of freshwater to the Atlantic Ocean on North America's eastern coast. The river discharge varies annually over a large range from >1500 $m^3 s^{-1}$ (in spring) to <70 $m^3 s^{-1}$ (in fall) (see insert in Fig. 3.1). Along the Altamaha estuary, diverse plant communities, including large expanses of tidal swamps and marshes as well as hardwood forest, are well established (Schubauer and Hopkinson, 1984). The Altamaha estuary and adjacent area are very productive, with annual primary production in a range of 600-700 g C $m^{-2} y^{-1}$ (Verity et al., 1993).

Sediment samples were collected from nine sites across the longitudinal transect in the Altamaha River in March 2002 and October 2002 (Fig. 3.1). The water salinities varied from nearly zero at the most upstream site (station A) to >30‰ at the mouth, with a slow gradient change in March (Fig. 3.2a). Sediment samples were collected using a box core sampler and the top 2 cm of sediment was immediately scraped from the core and stored frozen at -20°C until analyzed. The sediments collected within river sites (sites A to G) were dominated by <63 μm particles (clay and silt) while the sediments at site I (off the mouth) were dominated by sand (>63 μm) during both sampling periods (Fig. 3.2b). At site H (near the mouth), the relative proportions of sediment particles varied from silt/clay in March to sand in October (Fig. 3.2b).

Bulk parameter analysis. Total organic carbon (TOC), total nitrogen (TN), $\delta^{13}C_{TOC,}$ and $\delta^{15}N_{TON}$ were determined using a Carlo Erba CHN analyzer coupled to a Finnigan Delta C Stable Isotope Mass Spectrometer. To remove carbonate from sediments, ~0.5 g thawed sediment was treated with 10% HCl solution and sonicated in a water bath for 5 min. After air-drying in a fume hood followed by freeze-drying, the samples were ground up for elemental and bulk isotope analyses.

Pigment analysis. Pigments were extracted from ~1 g thawed sediment with 5 ml HPLC-grade 100% acetone three times. Combined extracts were filtered through a 0.2 μm GD/X polyethersulfone syringe filter (Whatman) and stored at -20°C for pigment analysis within two days. Chl-a concentration in sediment samples was determined by an ion-pairing reverse-phase high-performance liquid chromatography system (1100 series, Hewlett-Packard) with a quaternary pump and a variable wavelength detector. The column was a 5-μm C-18 (ODS) column (250 × 4.6 mm i.d., Alltech). Detection was accomplished by measuring absorbance at a wavelength of 420 nm. A gradient program ramped from eluant A (80% methanol; 20% aqueous solution of 0.5 mM tetrabutyl-ammonium acetate and 10 mM ammonium acetate) to eluant B (20% acetone in methanol) in 10 min with a hold time of 35 min. Identification of Chl-a was confirmed by co-injection with an authentic Chl-a standard (Sigma Chemical). The relative standard deviation of Chl-a analyses was less than ±3%, based on a series of daily measurements of the standard.

Lipid analysis. Lipid extraction and separation were based on a published procedure (Sun et al., 1999). In brief, ~2 g of thawed sediment was first extracted with 15 ml methanol followed by 3 × 15 ml mixed solvents (methylene chloride/methanol, 2:1 v/v). The lipids in combined extracts were defined as the "free" pool (solvent extractable). Lipids in this pool were saponified

using 0.5M KOH/MeOH to separate neutral and acidic lipids by hexane extraction under the basic condition (pH > 13, for neutrals) and acidic condition (pH < 2, for fatty acids). The extracted sediment residue was subsequently saponified with 0.5 M KOH/MeOH and the released lipids were defined as "bound" pool. The neutral and acidic lipids in the bound pool were separately extracted out with hexane under different pH conditions, as for the procedure for free lipids. Neutral lipids were treated with BSTFA [N,O-*bis*(trimethylsilyl)trifluoroacetamide] in acetonitrile to form TMS-ethers while acidic lipids were methylated with 14% BF_3-MeOH to form fatty acid methyl esters (FAMEs).

All lipids were analyzed by capillary gas chromatography using a Hewlett-Packard 6890 gas chromatograph with an on-column injector and a flame ionization detector. Lipid compounds were separated with a 30 m × 0.25 mm i.d. HP-5 column (0.25 μm film thickness). The temperature program was: 50–150°C at 20°C min^{-1}, followed by 150-310°C at 4° C min^{-1} and a hold at the final temperature for 5 min (for FAMEs) or 15 min (for neutral lipids). Internal standards [5α(H)-cholestane for neutral lipids and nonadecanoic acid methyl ester for FAMEs] were added to samples immediately prior to GC analysis to aid in quantification. The relative standard deviation of lipid analysis was within ±5% based on duplicate measurements. Lipid compounds were identified from their mass spectra by analyzing a few selected samples using a Shimadzu QP-5000 GC-MS system.

Compound-specific isotope analysis. Stable carbon isotopic ratios of individual lipids were determined using a GC-combustion system linked to a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS). Lipid compounds were separated with a 30 m × 0.25 mm i.d. column (DB-5, J&W Scientific) and then combusted to CO_2 over CuO/Pt wires at 850°C. The isotopic compositions of CO_2 peaks were measured with the mass spectrometer operated at 10 kV

acceleration potential and by magnetic sector mass separation. The $\delta^{13}C$ values of individual compounds are expressed relative to the SFC CO_2 (99.999%) reference gas ($\delta^{13}C$ = -11.23‰ relative to PDB) and the relative standard deviation of IRMS was within ±0.88‰ for fatty acids and ±0.21‰ for neutral lipids, based on internal standard measurements (n = 18 for each). The $\delta^{13}C$ values of FAMEs and TMS-ethers were corrected for the extra carbon atoms added by derivatization.

Modeling of bulk parameters. In our previous study (Dai et al., 2005), we analyzed chemical and isotopic compositions of three end-member organic materials (*Skeletonema costatum*, marine diatom; *Festuca arundinacea*, land grass; and *Spartina alterniflora*, salt marsh plant). Even when these organic materials degraded substantially, their original $\delta^{13}C$ and C/N ratios only varied over a small range (0.2–2‰ for $\delta^{13}C$ and 0.5–2.3 for C/N) and thus each remained distinctive. In contrast, the $\delta^{15}N$ ratios of three end-member materials varied over a relatively larger range (3–7‰) and overlapped each other. Thus, $\delta^{13}C$ and C/N ratios were chosen for modeling. We assume that organic materials in the sediments are partially degraded (50% fresh and 50% aged), so that average values of fresh and aged end-member organic materials were used. The relative proportions of organic matter derived from three typical sources were calculated by the following equations:

$$(C/N)_s = f_1 \times (C/N)_1 + f_2 \times (C/N)_2 + f_3 \times (C/N)_3$$

$$(\delta^{13}C)_s = f_1 \times (\delta^{13}C)_1 + f_2 \times (\delta^{13}C)_2 + f_3 \times (\delta^{13}C)_3$$

$$f_1 + f_2 + f_3 = 1$$

where f is the relative fraction of organic matter from different sources, and the subscripts s, 1, 2, 3 denotes sediment sample, fraction 1 (marine phytoplankton), fraction 2 (land plant), and fraction 3 (salt marsh plant) respectively.

Principal component analysis of biomarkers. To reduce the complexity of the biomarker data set (18 samples, two lipid pools, and 35 lipid compounds), we conducted PCA to examine distribution patterns of organic matter in Altamaha estuarine sediments. Two aspects of PCA were performed: (1) variable loadings, which represent correlation coefficients between each variable and principal component, and (2) PC scores, which indicate the relative influence of each PC on the sample composition. The weight percent normalized biomarker data were analyzed using STATISTICA' 98 Edition software. For technical simplicity, we assumed that the long-chain (C_{20}–C_{30}) even-number saturated fatty acids and alcohols are derived from terrigenous source (Meyers, 1997) and behave similarly towards different principal components during data analysis. Therefore, these compounds are grouped as individual variables (LCFA and LC-alc) respectively. Similarly, short-chain (C_{12}–C_{18}) even-number saturated fatty acids and alcohols are grouped as individual variables (SCFA and SC-alc) because they are ubiquitously distributed and thus non-specific in origin (Cranwell, 1982). In addition, branched *iso*-, *anteiso*-fatty acids, odd-number (15:0 and 17:0) fatty acids and 18:1(n-7) were grouped as one variable (BSFA) to represent bacteria-specific compounds (Kaneda, 1991). Other compounds (monounsaturated and polyunsaturated fatty acids, and sterols) were loaded as individual variables for PCA.

Results

Variations of bulk parameters. Relatively higher TOC (~4%) and TN (~0.3%) contents occurred in the sediments of upstream sites and these gradually decreased to near zero levels at the river mouth sites (Fig. 3.2c and Fig. 3.2d). At most sites, there were small variations in TOC (<1%) and TN (<0.1%) contents between two river discharge periods. However, at site H, both

TOC and TN contents dramatically decreased from March (high discharge) to October (low discharge), accompanying the shift in sediment type from silt/clay to sand. The C/N ratios of sediments generally varied over a range bordered by the ratios of C3 marine phytoplankton and C3 land plant (Fig. 3.2e). However, at a few sites (especially at D), C/N ratios were higher than the ratios found in C3 land plants.

In contrast to the variations in TOC, $\delta^{13}C_{TOC}$ ratios gradually increased from the upstream sites to the river mouth, mostly in the range between C3 land plant and C3 marine phytoplankton (Fig. 3.2f). There was little change in $\delta^{13}C$ ratios between two discharge periods at most sites, except at site D where the $\delta^{13}C_{TOC}$ in October was unusually enriched, even higher than that of C3 marine phytoplankton. On the other hand, $\delta^{15}N_{TN}$ ratios showed little variation along the transect between the two discharge periods except at site D, where the $\delta^{15}N_{TN}$ value was unusually depleted in October (Fig. 3.2g).

Distributions of biomarkers. Twenty-three fatty acids (saturated, monounsaturated, polyunsaturated, and branched) and twelve neutral lipid compounds (alcohols and sterols) were identified in Altamaha sediment samples. The contents of total fatty acids and neutral lipids (both free and bound) in the sediments varied spatially and temporally (Fig. 3.3): higher in March than in October, with several peaks along the transect. In March, the peaks occurred at sites B, E, and H while in October, the peaks shifted to C and G sites. These occurrences of peaks in lipid contents at different sites in high and low discharge periods were consistent with variations of Chl-a peaks in the sediments.

We classified the fatty acids and neutral lipids into terrestrial, algal, bacterial and mixed subgroups (Table 1). Different subgroups showed different distribution patterns in two discharge periods (Fig. 3.4). In the high discharge period (March), the concentrations of terrestrial fatty

acids and neutral lipids in sediments were higher at upstream sites A and B and decreased progressively towards the river mouth. During low discharge (October), the concentrations of terrestrial fatty acids and neutral lipids were generally low. Unlike the terrestrial lipid distributions, other three lipid subgroups (algal, bacterial, and mixed) showed similar distribution patterns, with three peaks at sites B, E and H in March, and two peaks at sites C and G in October. All these peaks occurred concomitantly with Chl-a peaks in both periods. Although concentrations of algal lipids and bacterial fatty acids were markedly lower at the peak sites in October relative to those in March, mixed lipids remained at similar concentrations in the two periods.

$\delta^{13}C$ compositions of lipid biomarkers. In this study, we presented $\delta^{13}C$ data of four lipid compounds (14:0, 16:0, and 24:0 fatty acids, and phytol) in the free pool because (1) they (except 24:0) are abundant lipid components in all samples and thus ensure a reliable isotope measurement; and (2) they have multiple sources each with a distinct isotope signature (Dai, et al., 2005). In both discharge periods, these lipid compounds were depleted (~-5‰) in ^{13}C relative to those of the bulk organic carbon at all sites in the estuary (Fig. 3.5). Generally, the $\delta^{13}C$ ratios increased from the upstream to the river mouth (except 24:0 fatty acid) in both discharge periods. However, like the variations of bulk $\delta^{13}C_{TOC}$, unusually enriched ^{13}C ratios of these lipid compounds, especially 24:0 fatty acid, were observed at station D in October.

The $\delta^{13}C$ ratios of two typical bacteria-specific fatty acids (*iso*- and *anteiso*-15:0) in the free lipid pool were chosen to examine the bacterial response to variable organic matter inputs in the two discharge periods. *Iso*-15:0 and *anteiso*-15:0 fatty acids in the sediments were relatively depleted in ^{13}C (-1 to -7‰) than those of bulk TOC (Fig. 3.6). In the high discharge period, the $\delta^{13}C$ values of these two bacteria-specific fatty acids varied over a small range from upstream to

the river mouth sites, and did not follow the pattern of the bulk $\delta^{13}C_{TOC}$. In the low discharge period, however, their variations followed the same trend as that of bulk $\delta^{13}C_{TOC}$. Especially, when the $\delta^{13}C_{TOC}$ unusually enriched at site D, the $\delta^{13}C$ ratios of the two bacteria-specific fatty acids appeared to coincide with the shift in $\delta^{13}C_{TOC}$.

Proportions of organic inputs into the sediments (modeling results). The modeling results (Fig. 3.7) indicate that C3 terrestrial plants and marine phytoplankton dominated the sedimentary organic matter (>80%) at most sites during high and low discharge periods, while C4 salt marsh plants contributed a >40% fraction of total organic carbon at site D during low discharge period. Fractions of phytoplankton gradually increased from the upstream sites to the mouth sites in October (low discharge), but more fluctuation occurred in March (high discharge). On average, the fraction of C3 terrestrial plants in the entire transect decreased by ~4% while the fraction of C3 phytoplankton increased by ~7.5% from March to October.

PCA results. The eigenvalue can be used as a criterion for determining appropriate factor numbers in PCA. Generally, the higher the eigenvalue, the more variance can be accounted for by the corresponding factors (Reemtsma and Ittekkot, 1992). When the eigenvalue is less than 1, the factor is less useful for interpreting the data set than the original variables. From our data set, the estimated eigenvalues of the three factors (PC1, PC2, and PC3) were 7.40, 3.84 and 2.12, respectively. PC1 and PC2 together represented 66% (43.5% and 22.5%, respectively) of the variability while PC3 covered only 12.5% of the variability. Thus, we selected PC1 and PC2 to interpret the geochemical relationship between lipids and samples.

The first step of PCA is to plot the variable loadings of PC1 versus PC2 (Fig. 3.8a), which reflects the correlation between each variable (individual or group biomarkers) and each PC. PC1 is most negatively loaded (loading <-0.85) on individual polyunsaturated fatty acids (20:4 and

22:6) and individual sterols ($27\Delta^{5,22}$, $27\Delta^{5}$, and $28\Delta^{5,22}$). These compounds have been commonly attributed to fresh and labile plankton materials (Volkman, 1986; Mannino and Harvey, 1999). The strong loadings of two polyunsaturated fatty acids also indicate that PC1 represents an aspect of OM lability as these compounds are readily degraded (Zimmerman and Canuel, 2001). In contrast, the compound group with the most positive loading on PC1 is SCFA (0.72), which is generally from a variety of sources (Cranwell, 1982) and tends to be more geochemically stable than the negatively loaded compounds (Zimmerman and Canuel, 2001). PC2 is most positively loaded (loading >0.70) on LCFA and LC-alc, representing terrigenous/allochthonous input (Meyers, 1997), and most negatively loaded (loading <-0.65) on $28\Delta^{5}$ and phytol, indicating autochthonous input (Volkman et al., 1989). The BSFA group of bacteria-specific fatty acids is negatively loaded on PC1 (loading -0.59), but its PC2 loading is slightly negative (-0.16), implying that bacteria primarily utilize fresh and labile organic matter, which may come from either allochthonous or autochthonous source (Ittekkot and Lanne, 1991).

The second step of PCA is to confirm the relative influence of each PC on the sample composition by plotting scores of PC1 versus PC2 (Fig. 3.8b). Samples collected in March (high discharge) and October (low discharge) are clearly distinguished on the PC score plot. Almost half of March samples (B, E, F, and H) have negative PC1 scores, while all October samples have positive scores on PC1. On the other hand, PC2 scores of March samples are generally more positive than those of October samples (an exception is sample D in October).

Discussion

Variations of organic matter sources based on bulk parameters. Although there were only little or small variations in TOC and TN contents between the two discharge periods at most sites

in the Altamaha estuary, remarkable decreases of TOC and TN from March to October were observed at site H. An obvious cause for the changes is likely related to the shift in sediment type at this river mouth site. It is well known that the organic carbon and nitrogen contents of muddy sediments are generally higher than those in sandy sediments (Mayer, 1994; Keil and Cowie, 1999). The spatial variations of TOC and TN along the salinity gradient in the Altamaha River seem to be related to the distributions of sediments with higher contents at upstream sites where the sediments are dominated by silt/clay and gradually decreasing towards the mouth when the sediments became sandier.

The C/N ratios of sediments in the Altamaha estuary decreased from the upstream sites to the river mouth sites, indicating a shift of organic matter input from different sources. At most upstream sites (A to C), the C/N ratios of sediments were close to that of land plant while at the mouth sites (H and I), the C/N ratios approached to that of marine phytoplankton, reflecting the varying relative importance of terrestrial versus marine organic inputs between the two parts of the estuary. However, the transition from terrestrial higher plant input at the upstream sites to marine phytoplankton input at the mouth sites was not smooth as indicated by the unusually higher C/N ratio at site D, possibly reflecting salt-marsh inputs.

A shift of organic matter input from terrestrial source to marine source is also indicated by the variations of bulk $\delta^{13}C_{TOC}$. Similar $\delta^{13}C$ distribution patterns have been observed in many estuarine systems (Cai et al., 1988; Fogel et al., 1992). Although $\delta^{13}C_{TOC}$ ratios varied little between the two discharge periods at most sites, the $\delta^{13}C_{TOC}$ at site D was unusually enriched in October. The reason for this enrichment is likely related to a substantial input from C4 salt marsh plant, consistent with unusually higher C/N ratio in the sediment. Small differences in $\delta^{13}C_{TOC}$ between two discharge periods at most sites also suggest that the source rather than diagenetic

processes is a primary factor controlling the $\delta^{13}C$ distribution in the estuarine sediments.

The organic matter of estuarine end members have distinct $\delta^{15}N$ signatures (Peters et al., 1978; Wada and Hattori, 1978), but when fresh organic materials degrade their $\delta^{15}N$ ratios can vary positively or negatively over a range of 4-7‰ (Lehmann et al., 2002; Dai et al., 2005). The enrichments in $\delta^{15}N_{TN}$ during degradation are likely caused by preferential release of ^{15}N-depleted dissolved components (Ostrom et al., 1997; Sachs and Repeta, 1999) while the depletions may be due to the selective removal of ^{15}N-enriched proteins (Macko et al., 1987). Moreover, the addition of ^{15}N-depleted organic matter from bacterial growth may lead to a decline in $\delta^{15}N$ (Lehmann et al., 2002). Similar to the occurrence of anomalous C/N and $\delta^{13}C_{TOC}$ ratios at site D in October, the $\delta^{15}N_{TN}$ of this sediment (site D) in October was quite different from those at other sites, reflecting the complexities of organic matter inputs and biogeochemical processes.

Distributions of organic matter in the sediments based on biomarkers. Examination of lipid biomarker distributions also provides an insight into specific sources of organic matter in estuarine environments (Canuel et al., 1995; Sun et al., 2000). Higher amounts of total lipids were found in March than in October at most sites even though the amounts of TOC were similar, implying that organic matter deposited at different times might have experienced different biogeochemical processes. Occurrence of lipid peaks in the sediments coincided closely to those of Chl-a, suggesting that algal input might be one major contributor of lipids into the sediments. An exception was at site A in March: when Chl-a was at very low level, total fatty acids and neutral lipids remained at considerably higher levels, indicating that algal input at this upstream site might not be an important contributor to total lipids.

Spatial and temporal variations of terrestrial lipids (long-chain saturated fatty acids and

alcohols) indicate that: (1) input from land-derived organic matter is relatively more important in the high discharge period than in the low discharge period; and (2) a large fraction of terrestrial organic matter deposits at the upstream sites of the river when freshwater meets seawater there. In contrast, the higher concentration of algal lipids at particular sites varied from March to October. Unlike terrestrial organic matter, whose deposition is largely controlled by initial mixing of freshwater and seawater, the deposition of algal organic matter is closely related to the locations of enhanced algal productivity.

The close correlation between algal lipids and bacteria-specific fatty acids indicates that bacteria are largely dependent on organic matter from algal source. A similar correlation was observed in Altamaha estuarine sediments in the 1998 high discharge period (Sun et al., 2000). On the other hand, mixed lipids also followed the same distribution patterns as algal lipids in the two discharge periods, implying that a proportion of algal input in this mixed pool may be relatively more important than other inputs. From lipid composition analysis of end-member materials (Dai et al., 2005), fresh land-plant and salt marsh plant contained abundant polyunsaturated 18:2 and 18:3 fatty acids, but they mostly disappeared when the materials degraded. There were little 18:2 and 18:3 fatty acids to be found in all sediment samples collected in all sites and in two discharge periods, suggesting that organic matter from terrestrial and salt marsh plants are largely degraded before they deposited into the sediments.

Compared to $\delta^{13}C_{TOC}$ ratios (Fig. 3.2f), the $\delta^{13}C$ ratios of four lipid compounds (14:0, 16:0, 24:0 fatty acids and phytol) were relatively depleted (~-5‰) in all sites. This is due to the fact that lipid compounds are generally depleted in $\delta^{13}C$ (-3 to -6‰) compared with bulk organic matter (Hayes, 1993; Boschker et al., 1999). The variation patterns of compound-specific $\delta^{13}C$ ratios were coincident with those of bulk $\delta^{13}C$ (depleted values in upstream sites and an

enrichment towards the mouth sites), suggesting a shift of organic matter input from terrestrial sources to algal sources. However, unusually enriched $\delta^{13}C$ ratios of lipid compounds (especially 24:0 long-chain fatty acid, a common compound of land plants and salt marsh plants but not marine phytoplankton) occurred at site D in low discharge period, reflecting a significant organic input from salt marsh plants.

Synthesized picture of organic matter sources based on modeling and PCA. For estuaries where upland plants and phytoplankton are the major sources of organic matter, the relative contributions of organic matter have been generally assessed by a two-end-member model (Eadie et al., 1994). In this study, we applied a three-end-member model based on a consideration that C4 salt marsh plants may be an important OM contributor in the Altamaha estuary. There is still a limitation in the model application due to a lack of information on other potential OM sources such as soil-derived materials, benthic microalgae, and freshwater phytoplankton. A critical requirement for end-member modeling is the invariability of signatures during degradation of organic matter. For example, C/N ratios have been variously observed to either increase, decrease, or show no change during diagenetic processes (Rosenfeld, 1979; Henrichs and Farrington, 1987). From our previous study (Dai et al., 2005), however, C/N and $\delta^{13}C$ ratios of three end-member organic materials (land grass, salt marsh plant, and marine diatom) varied over small ranges (0.5 to 2.3 for C/N and 0.17 to 1.99‰ for $\delta^{13}C$) when these materials degraded by 20–60%. In our calculations we have assumed that the organic matter in the sediments consists of partially-degraded (50%) materials based on a consideration that little 18:2 and 18:3 fatty acids (important compounds in fresh plants) are present. Thus, we can use averaged C/N and $\delta^{13}C$ ratios from fresh and degraded end materials as baselines for modeling.

The first view revealed by the modeling is that organic matter in Altamaha estuarine

sediments is dominated by C3 terrestrial plants and phytoplankton (~80%) at most sites while C4 salt marsh plants contribute a large fraction (~40%) into the total organic carbon pool only at one site (D) in the low discharge period. The occurrence of peak salt marsh input at site D in October is attributed to several causes. The first is the timing of washout of salt marsh plants into the river after die-off during fall (Wiegert et al., 1981). The second is the effect of seawater intrusion on transport of salt marsh-derived detritus during low discharge (Brockway et al., 2006). The third is the geographic feature of site D in the estuary (Fig. 3.1), where the river bends and may block the inland transport of salt marsh-derived detritus.

Secondly, the relative contributions of terrestrial organic matter versus phytoplankton varied between the high and low discharge periods. Fractions of terrestrial organic matter and phytoplankton progressively (with an exception at site D due to a large input from salt marsh plants) changed from the upstream to the mouth in the low discharge period while there were several fluctuations in their relative fractions in the high discharge period. A possible cause for these different distributions is linked to the changes in mixing regimes between the two periods. During low discharge, seawater and freshwater are more fully mixed (Dyer, 1997) resulting in a relatively conservative deposition of organic matter from different sources. In contrast, during the high discharge period, seawater and freshwater are partially mixed, resulting in a stratified water column (Dyer, 1997). Deposition of particulate organic matter in the stratified water may be non-conservative. In addition, seasonal variation of phytoplankton production in estuarine water may affect the relative deposition of organic matter from different sources. Generally, the primary production rate in this area is at a minimum in winter and early spring when discharge is high, but the rate reaches the maximum in summer when the discharge is low (Verity et al., 1993).

The biomarker PCA results show that in the high discharge period, organic matter at most sites is dominated by allochthonous sources while in the low discharge period, autochthonous organic matter is a major fraction at most sites (except site D due to a large input from salt marsh plants). This pattern is consistent with the distributions of terrestrial lipids in two discharge periods: higher % of long-chain fatty acids and alcohols (relative to the total lipids) in March than in October at most sites. However, the distributions of algal lipids in two discharge periods do not follow this pattern: either higher or lower % of algal lipids (relative to the total lipids) in March or October. The inconsistency between PCA pattern and algal lipid distributions can be explained by relative variations of degradation processes between two periods. In summer and fall (low discharge), although phytoplankton production is greater, labile organic compounds are largely degraded in water and sediments due to faster metabolic processes at high temperatures (Canuel and Martens, 1996). Moreover, algal lipids (e.g., polyunsaturated fatty acids) are readily degraded compared to terrestrial lipids (e.g., long-chain saturated fatty acids) due to the structural feature (Harvey et al., 1997). Therefore, the content of algal lipid compounds in sediments can be low despite a large fraction of organic matter from aquatic production. On the other hand, at several sites (B, E, F, and H), organic matter deposited in the high discharge period seems to be fresher than those in low discharge period. This can be attributed to slow degradation processes at low temperature in March.

Use of organic matter by bacteria. Many studies have demonstrated that bacterial utilization of organic carbon is controlled by bioreactivity of different organic matter fractions (Hopkinson and Vallino, 1995; Smith and Hollibaugh, 1995). In this study, we observed that distributions of bacteria-specific fatty acids in Altamaha estuarine sediments varied coincidently with those of algal lipids and Chl-a in both discharge periods, suggesting that input of labile

organic matter from aquatic algae may control the microbial metabolism (Kemp, 1990).

In the high discharge period, $\delta^{13}C$ ratios of bacteria-specific fatty acids varied over a relatively small range (~2‰) from the upstream to the river mouth sites, despite bulk $\delta^{13}C_{TOC}$ shifted progressively from ~-26 to ~-20‰ along the transect. Different variation patterns of $\delta^{13}C$ ratios between TOC and bacteria-specific fatty acids indicate that the organic carbon utilized by microbial community in the sediments may be largely from algal sources. As shown by the end-member modeling, the organic matter during this high discharge period was dominated by C3 terrestrial and algal inputs. Although deposited organic matter in the sediments shifted from dominating terrestrial input at upstream sites to dominating marine algal source at river mouth sites, bacteria might largely utilize labile organic carbon from algal source.

In the low discharge period, $\delta^{13}C$ ratios of bacteria-specific fatty acids in the sediments followed closely the variations of $\delta^{13}C_{TOC}$, which is different from the pattern in the high discharge period. Especially, at site D, there were coincident occurrence of unusually enriched $\delta^{13}C$ ratios of both TOC and bacteria-specific fatty acids. A close correlation between $\delta^{13}C$ of bacteria-specific fatty acids and $\delta^{13}C$ of total suspended POC was observed in other temperate estuaries (Boschker et al., 2005), which is controlled by a coupling between primary production and bacterial consumption in lower marine side of the estuary and a link between terrestrial organic matter and bacterial growth. Changes in the $\delta^{13}C$ pattern of bacteria-specific fatty acids in Altamaha estuarine sediments from high to low discharge periods indicate a corresponding response of bacterial community to variations in organic matter input and environmental conditions. Although algal production is higher in fall than in spring, metabolism in water and sediments is more active due to higher temperature. Thus, algae-derived organic matter may be deposited in the sediments as less labile components (as shown by PCA), which hinders

utilization of this organic matter. When salt marsh-derived organic matter becomes a significant fraction of total organic carbon in the sediments, bacteria may utilize this carbon, resulting in an enriched $\delta^{13}C$ ratio in their specific fatty acids.

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Table 3.1. Lipid subgroups, abbreviations, and their potential sources.

Subgroups	Compounds	Abbreviation	Major sources	References
Mixed	short-chain even-number (C_{12}-C_{18}) saturated fatty acids	SCFA	non-specific	(1 – 7)
	short-chain even-number (C_{12}-C_{18}) saturated alcohols	SC-alc	non-specific	
	monounsaturated fatty acid 18:1(n-9)	18:1(n-9)	non-specific	
	phytol	phytol	algae, higher plants	
	cholest-5-en-3β-ol	$27\Delta^5$	zooplankton, algae, higher plants	
	24-methylcholest-5-en-3β-ol	$28\Delta^5$	algae, higher plants	
	24-ethylcholesta-5,22-dien-3β-ol	$29\Delta^{5,22}$	terrestrial higher plants, algae	
	24-ethylcholest-5-en-3β-ol	$29\Delta^5$	terrestrial higher plants, algae	
Algal	polyunsaturated fatty acids (22:6, 20:5, 20:4)	PUFA	fresh plankton	(4, 8 – 10)
	monounsaturated fatty acid 16:1(n-7)	16:1(n-7)	fresh plankton	
	cholesta-5,22-dien-3β-ol	$27\Delta^{5,22}$	algae	
	24-methylcholesta-5,22-dien-3β-ol	$28\Delta^{5,22}$	algae	
Bacterial	branched (iso- and anteiso-), odd-number (C_{15} & C_{17}), and 18:1(n-7) fatty acids	BSFA	bacteria	(11)
Terrestrial	Long-chain (C_{20}-C_{30}) saturated alcohols	LC-alc	terrestrial higher plants	(12)
	Long-chain (C_{20}-C_{30}) saturated fatty acids	LCFA	terrestrial higher plants	

References: (1) Cranwell, 1982; (2) Zimmerman and Canuel, 2001; (3) Prahl et al., 1984; (4) Volkman, 1986; (5) Mannino and Harvey, 1999; (6) Harvey, 1994; (7) Nichols et al., 1990; (8) Colombo et al., 1996; (9) Volkman et al., 1989; (10) Yunker et al., 1995; (11) Kaneda, 1991; (12) Meyers, 1997.

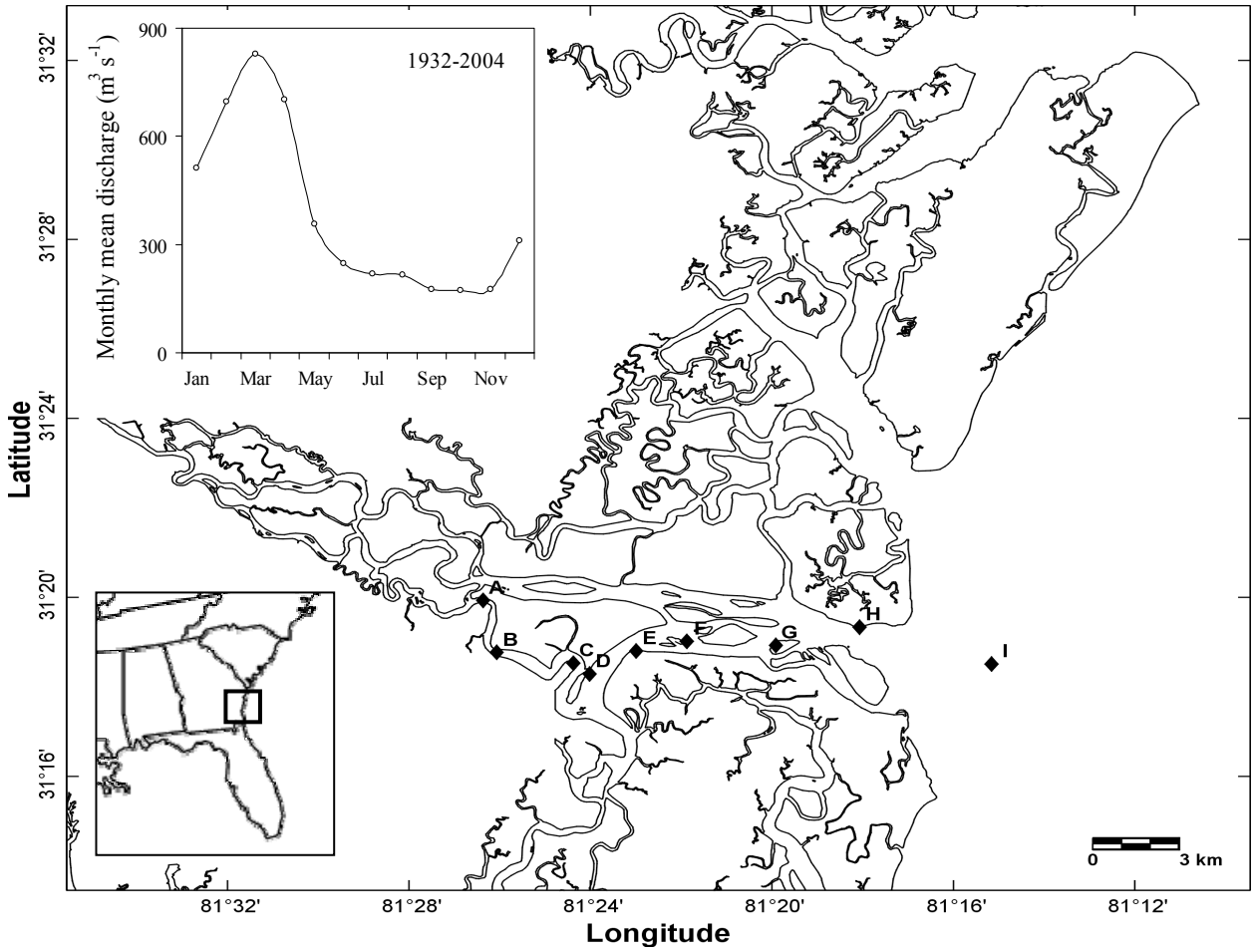

Fig. 3.1. Sampling stations (A to I) in the Altamaha River estuary during March and October 2002. (upper insert: monthly mean discharge from 1932 to 2004; bottom insert: Georgia coast.)

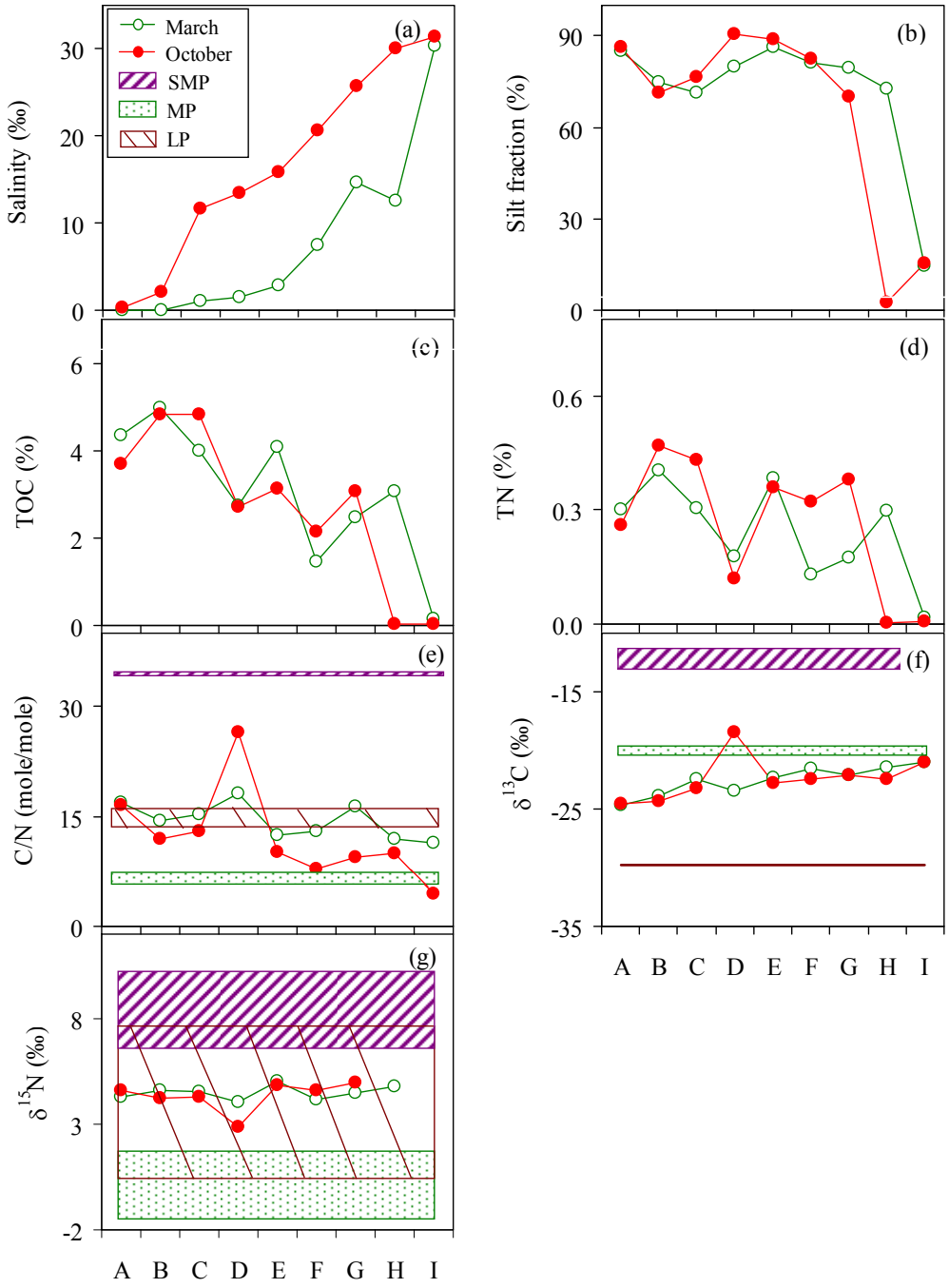

Fig. 3.2. Variations of bulk parameters (a) salinity, (b) silt fraction, (c) TOC, (d) TN, (e) C/N ratio, (f) $\delta^{13}C_{TOC}$, and (g) $\delta^{15}N_{TN}$ along the Altamaha River estuary during two discharge periods. Strips represent the ranges of bulk parameters of three end-member organic materials (fresh and degraded salt marsh plant, SMP; marine phytoplankton, MP; and land plant, LP).

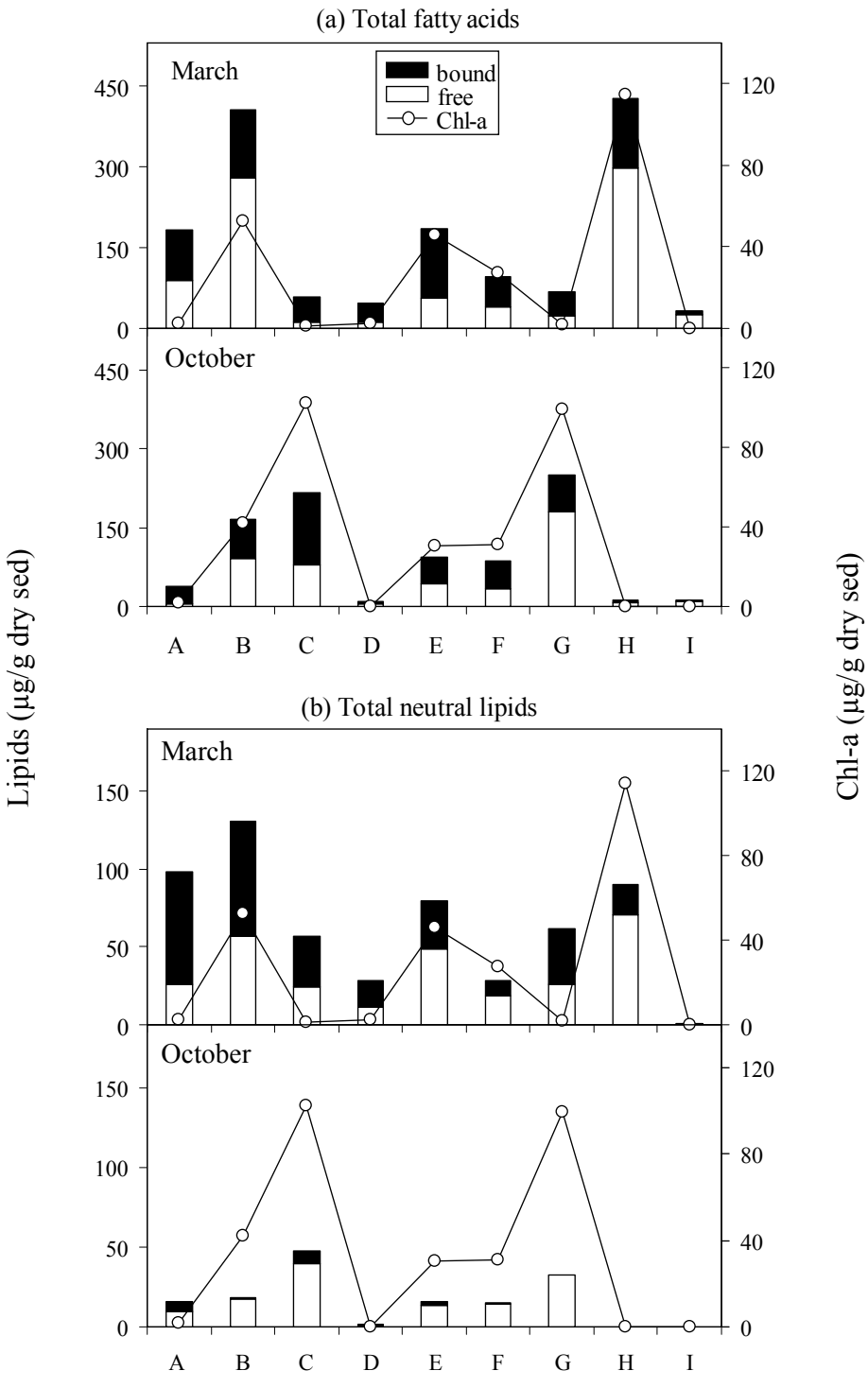

Fig. 3.3. Distributions of (a) total fatty acids and (b) total neutral lipids in surface sediments along the Altamaha River estuary during two discharge periods. Chl-a data are included.

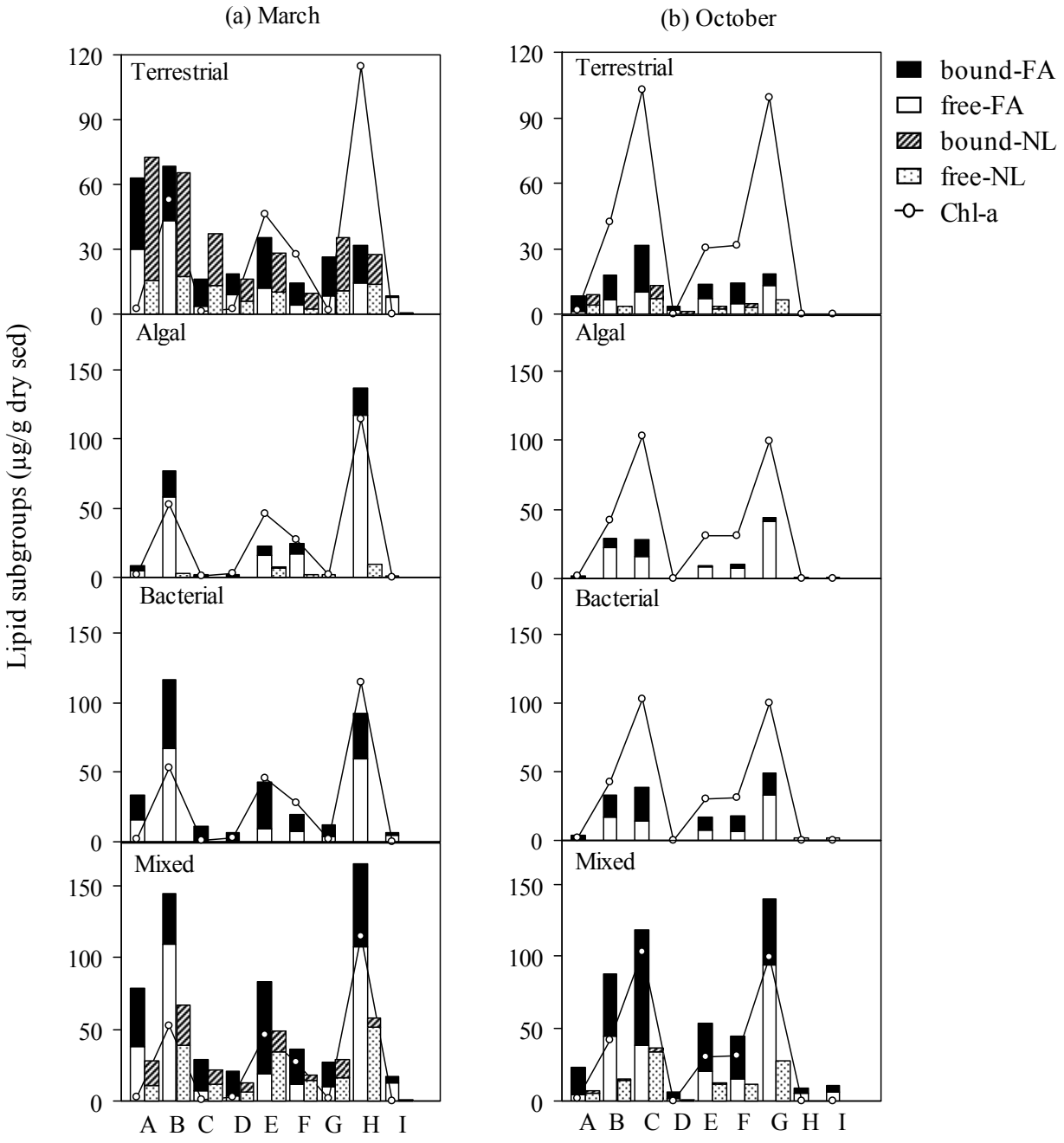

Fig. 3.4. Distributions of free and bound lipid subgroups (terrestrial, algal, bacterial, and mixed) in surface sediments along the Altamaha River estuary during (a) high discharge period and (b) low discharge period. (FA - fatty acid and NL - neutral lipid).

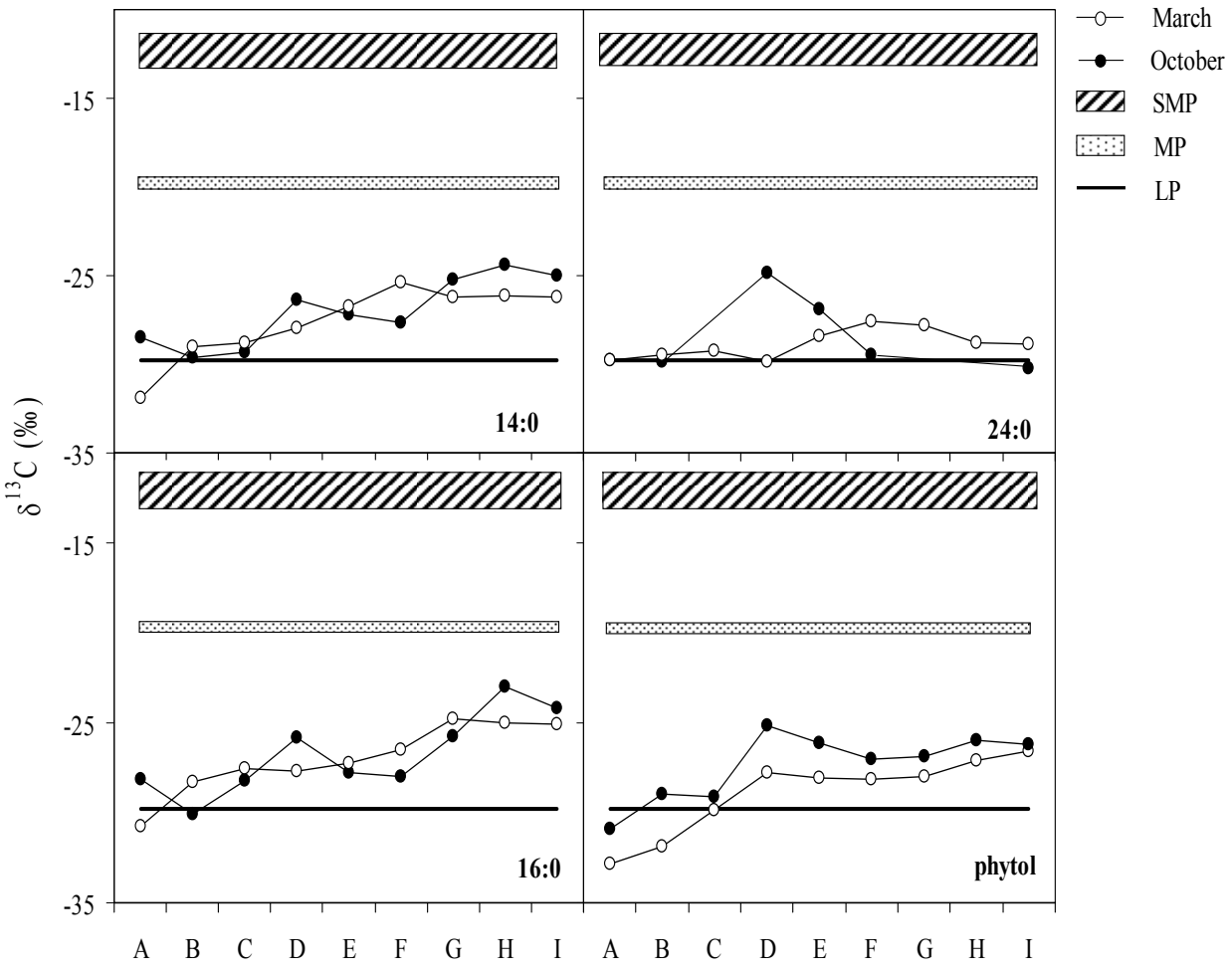

Fig. 3.5. Variations of $\delta^{13}C$ ratios of 14:0, 16:0, and 24:0 fatty acids, and phytol in surface sediments along the Altamaha River estuary during two discharge periods.

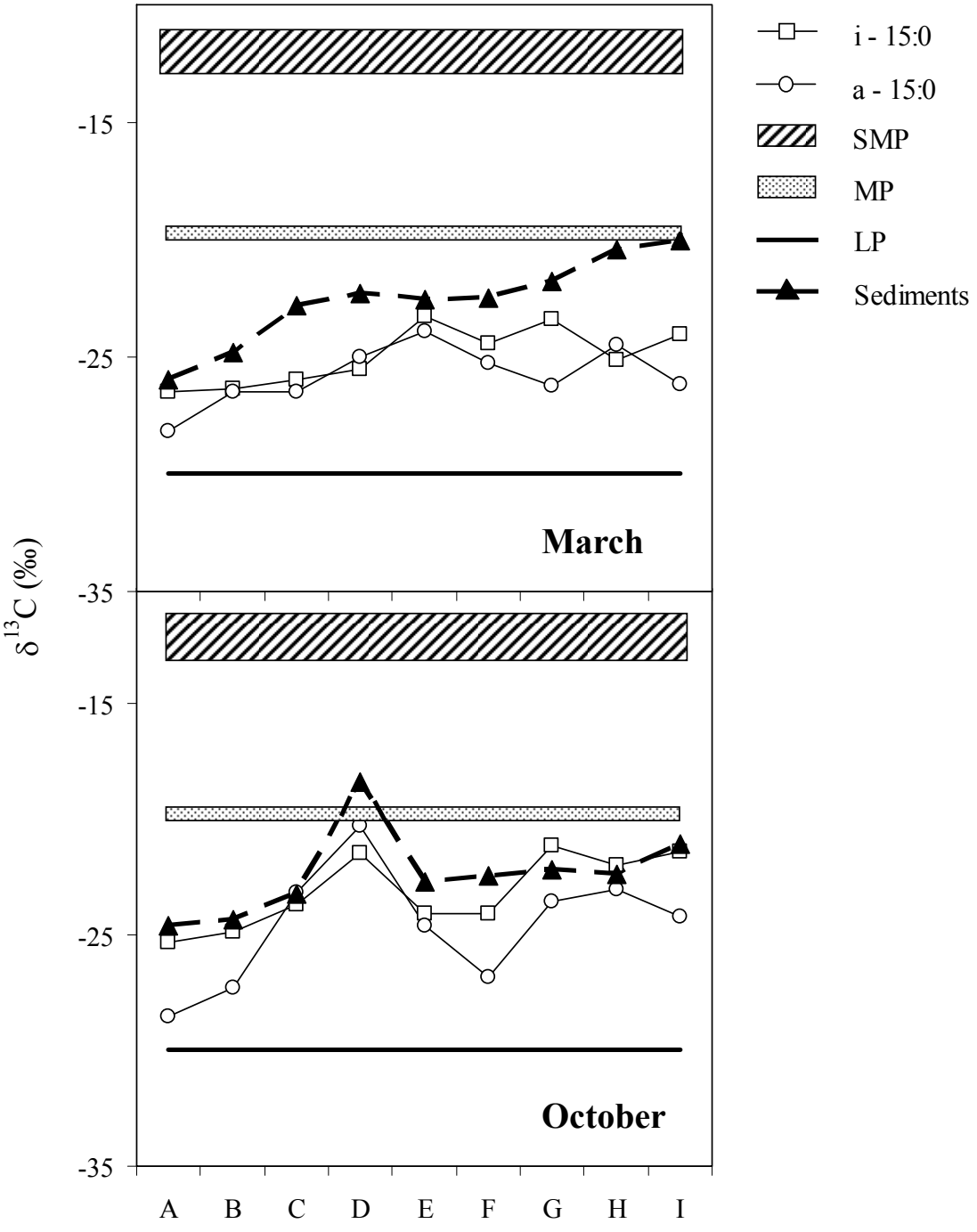

Fig. 3.6. Variations of $\delta^{13}C$ ratios of bacteria-specific fatty acids (*iso*-15:0 and *anteiso*-15:0) in surface sediments along the Altamaha River estuary during two discharge periods.

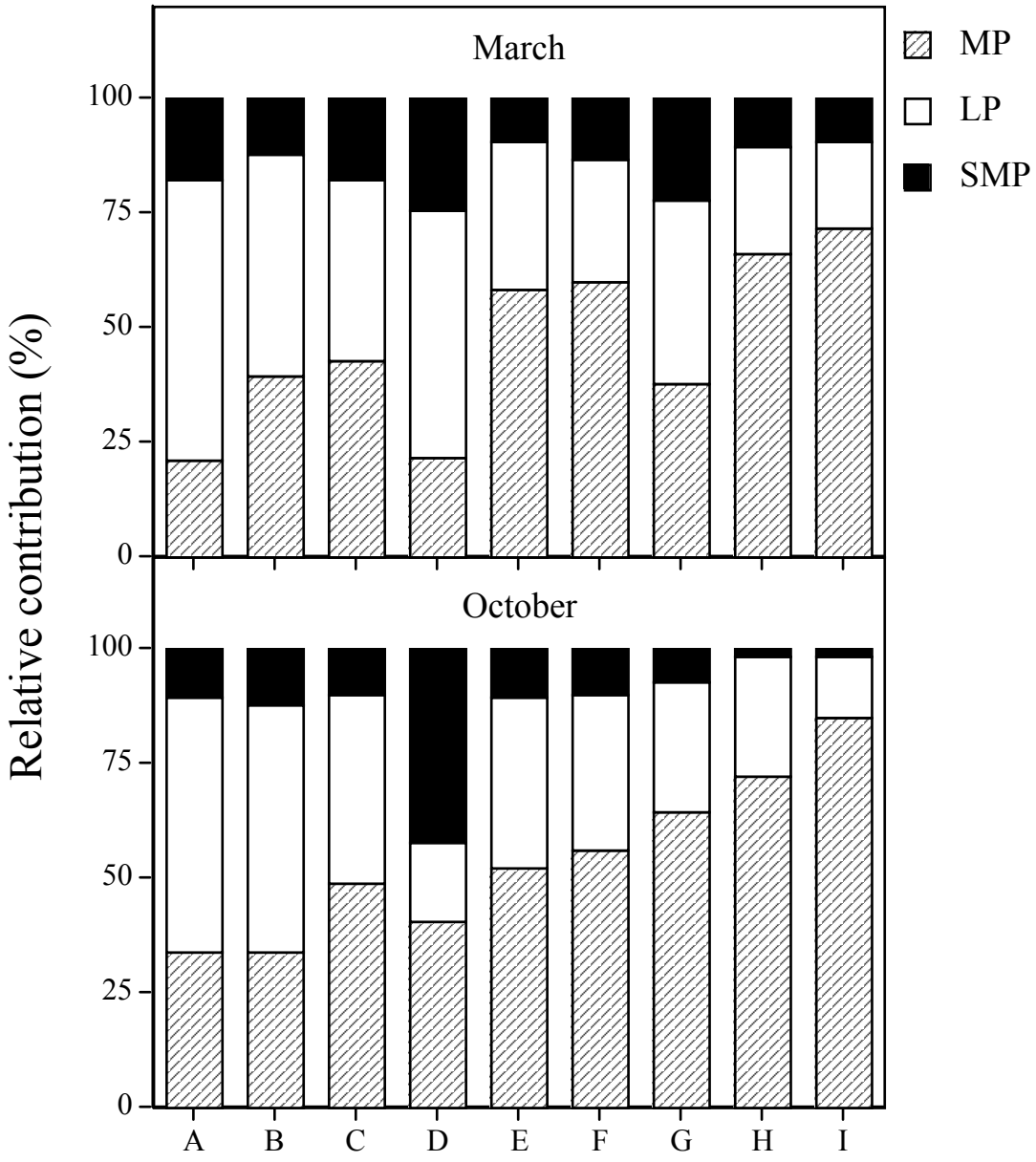

Fig. 3.7. Relative contributions (%) of organic matter from three major sources in surface sediments along the Altamaha River estuary during two discharge periods.

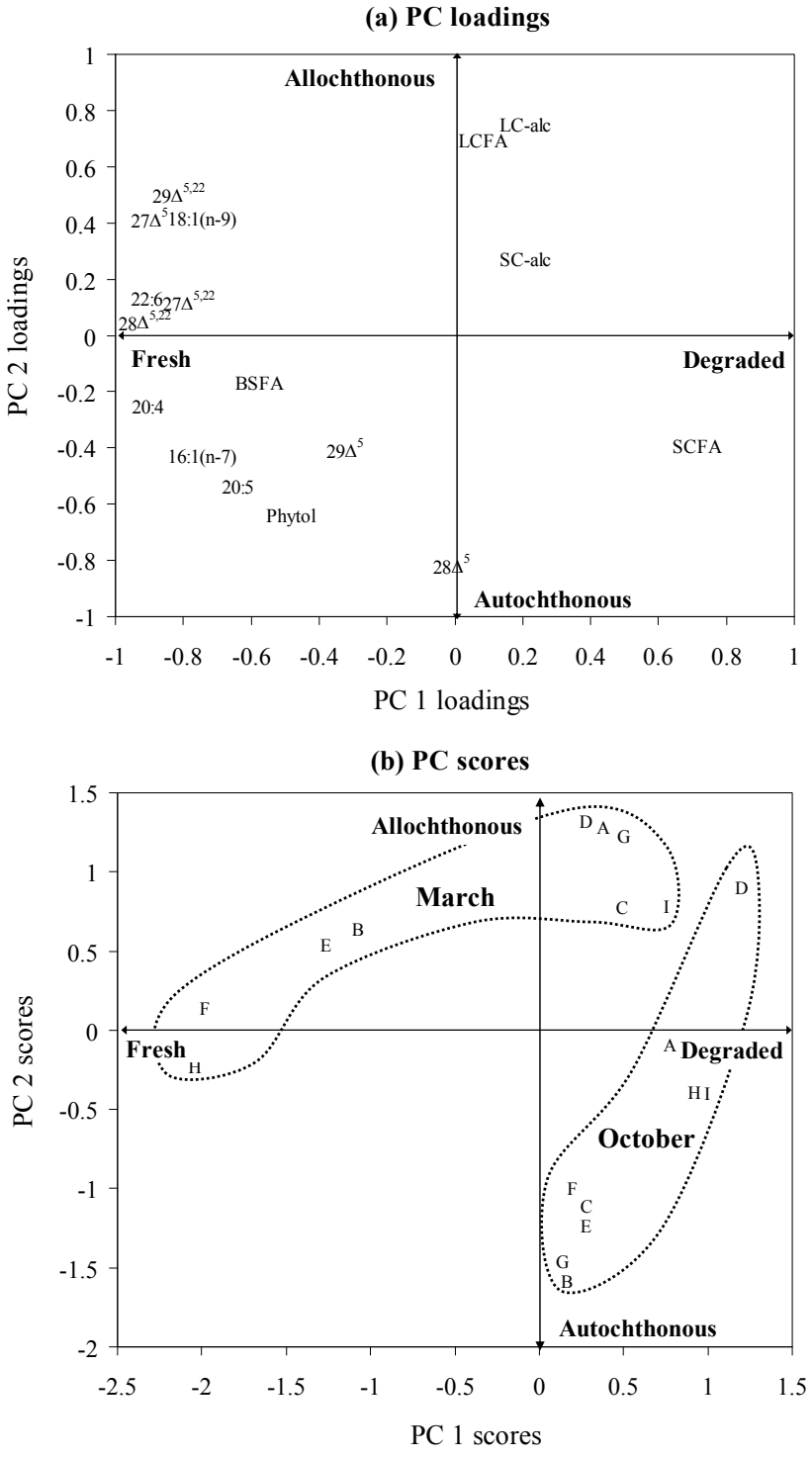

Fig. 3.8. (a) PC loadings of lipid biomarkers in surface sediments of the Altamaha River estuary (abbreviations are given in Table 1), and (b) PC scores for sediment samples.

CHAPTER 4

BIOCHEMICAL REACTIVITIES OF VARIOUS END-MEMBER ORGANIC MATERIALS AND THEIR UTILIZATION BY BACTERIA IN ALTAMAHA ESTUARINE SEDIMENTS

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Abstract

Organic matter in estuarine and coastal marine sediments originates from a variety of sources and is subject to intensive biochemical degradation before burial. This study experimentally determined the biochemical reactivities of organic materials from three major sources (marine phytoplankton, land grass, and salt marsh plants) by incubating fresh materials in sediments with different redox conditions. The substrate materials were either separately or together added to the pre-incubated (aged) sediments to test the effect of co-metabolism on organic matter degradation. We followed the variations of bulk parameters (TOC, TN, C/N ratio, $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$), fatty acids and their stable carbon isotopic compositions over three-month incubations. A comparison of degradation rate constants of TOC and fatty acids among different incubations indicated an order of biochemical reactivity for different materials: marine diatom > land grass > salt marsh plant. On the other hand, mixing of marine diatom with the land grass and salt marsh plant did not stimulate the overall degradation of organic matter in the sediments. The $\delta^{13}C$ variations of 16:0 fatty acid (non-specific) during incubations further confirmed that the residual organic matter after incubations was dominated by salt marsh plant. The ratios between oxic and anoxic degradation rate constants (k_{ox}/k_{an}) of TOC and most fatty acids (except 16:0 in salt marsh plant incubation) were in a range of 1.1 - 1.7, implying that redox conditions had a small influence on the degradation of fresh organic matter in the aged sediments. The variations of bacteria-specific fatty acids and their $\delta^{13}C$ ratios indicated that bacterial metabolism could utilize any fresh material available in the sediments when only a single organic material was added, but marine diatom was more preferentially utilized by bacteria in the sediments with addition of mixed materials.

Keywords: Estuarine sediments, Biochemical reactivity, Organic matter degradation, Bulk parameters, Fatty acids, Degradation rate constant, Redox conditions, Co-metabolism, Bacterial response

Introduction

There is increasing interest in the degradation of organic matter in estuarine and coastal marine systems due to its extremely important role in controlling global carbon cycling, particularly the preservation of organic matter in sediments (Wakeham and Canuel, 2006). Generally, aquatic plankton and terrestrial higher plants are the major inputs of organic matter into the sediments (Wiegert et al., 1981, Mulholland and Olsen, 1992; Canuel and Martens, 1993; Shi, et al., 2001). However, along the Southeastern coast of USA, extensive salt marsh plants contribute a large fraction of organic matter after dying-off during warm seasons (Haines 1976; Wiegert et al., 1981; Hopkinson, 1985; Peterson and Howarth, 1987). These end-member organic materials possess distinctly different chemical and isotopic signatures (e.g., TOC, TN, C/N ratio, bulk $\delta^{13}C$ and $\delta^{15}N$ ratios, biomarker and molecular isotopic compositions), which have been used to assess source, transport, alteration, and preservation of organic matter in estuarine and coastal marine systems (Fry and Sherr, 1984; Lajtha and Marshall, 1994; Fogel and Tuross, 1999).

When organic materials enter the sediments, they experience intensive degradation mediated by benthic communities. Many studies have shown that more than half of the particulate organic carbon and nitrogen, which are settled from the water column, can be degraded on the sediment-water interface (Hedges et al., 1988a, b; Kemp, 1990; Parkes et al., 1994). Generally, organic materials from different sources have different susceptibilities to

biochemical degrading agents. For example, marine-originated organic matter is more readily utilized by benthic community compared to that from upland higher plants because the latter contain higher content of biochemically resistant compounds such as lignin, resins, and waxes (Fenchel and Blackburn, 1979; Ittekkot and Lanne, 1991; Canuel and Martens, 1993). On the other hand, accumulating evidence has demonstrated that organic matter from terrestrial sources is rapidly removed once it enters estuarine and coastal marine systems (Hedges et al., 1988a, b; 1997), implying that metabolic consumptions of both autochthonous and allochthonous organic materials may be equally important in these systems (Hopkinson and Vallino, 1995; Smith and Hollibaugh, 1995). However, it has been unclear whether co-metabolism of labile and refractory organic matter is a major cause for rapid degradation of organic matter from all sources (Wakeham and Canuel, 2006).

Canfield (1994) proposed a pseudo-G model to establish relationships between refractory and labile organic matter. It suggests that degradation processes of labile and refractory organic matter may be inter-linked rather than independent, as suggested by the classic "multi-G model" (Jϕrgensen, 1978; Berner, 1980; Westrich and Berner, 1984). High metabolic activity resulting from the decay of labile organic matter probably enhances the decomposition of refractory organic matter (Canfield, 1994). However, few experimental studies have been conducted to test this hypothesis, especially at molecular level (Wakeham and Canuel, 2006).

Redox conditions appear to be another important factor affecting the biochemical degradation of organic matter in sediments but our understanding on the role of oxygen has been equivocal (Emerson and Hedges, 1988; Pederson and Calvert, 1990; Sun et al., 1993; Cowie et al., 1995; Canuel and Martens, 1996). The major concern is on whether or not organic matter is more efficiently degraded with presence of oxygen. Some studies have demonstrated that oxygen

has little influence on the degradation of organic matter in sediments (Henrichs and Reeburgh, 1987; Pederson and Calvert, 1990, Lee, 1992), whereas other studies have shown that aerobic degradation prevails over anaerobic degradation for many organic compounds (Sun et al, 1997, 2002; Teece et al., 1998; Hoefs et al., 2002). Canfield (1994) concluded that both contrasting arguments could be correct, depending on the deposition rate and its influence on the oxidation pathways of organic matter. It seems that high deposition rate results in a large input of fresh or labile organic matter into sediments where it degrades regardless of presence of oxygen. In contrast, the influence of redox conditions becomes more important for refractory materials in the sediments with a low deposition rate (Kristensen and Blackburn, 1987; Middelburg, 1991).

This experimental study was designed to determine the biochemical reactivities of three end-member organic materials in Altamaha estuarine sediments. We focused on the effects of co-metabolism and redox conditions on organic matter degradation by incubating different organic materials under variable conditions. We followed the variations of bulk parameters (TOC, TN, C/N ratio, $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$), fatty acid concentrations and compound-specific isotopic compositions over three-month incubations. The degradation rate constants of TOC and major fatty acids, which represent specific sources of different organic materials, were estimated to assess the relative influences of redox conditions and co-metabolism on organic matter degradation. In addition, we examined the responses of bacteria to varying organic matter supplies by monitoring the variations of bacteria-specific fatty acid concentrations and their $\delta^{13}C$ ratios in different incubation systems.

Experimental

Organic materials and sediments. Three typical end-member organic materials used in this

study are: (1) C3 marine diatom *Skeletonema costatum* (clone CCMP1332, the center for Culture of Marine Phytoplankton, Booth Bay Harbor, ME, USA), cultured in F/2 medium with a 12:12 light/dark cycle at 16ºC over 14 days; (2) C4 salt marsh plant (*Spartina alterniflora*), collected from Sapleo Island, Georgia, USA; and (3) C3 land grass (*Festuca arundinacea*), collected from the upland of Georgia. Seawater (salinity ~28‰) was collected from the mouth of the Altamaha River by pumping surface seawater through a set of filters (25μm). Sediments used in the experiments were collected from a site (31°18.27'N, 81°23.89'W) in the Altamaha River estuary by using a box core sampler. Top 2 cm of sediments were scraped from the core and stored in a covered container. The sediment was pre-incubated under room temperature over 6 months to minimize the content of labile organic matter.

Incubation setup. Experiments were conducted in "open" incubation systems (Fig. 1), where sediment plugs sit on the bottom of large seawater reservoirs (~10L). The sediment was passed through a 0.5 mm sieve to remove macrobenthos, large shells and detritus before the incubation. The sediment plugs (PVC plates with plastic rings, 1 mm thickness and 5 cm i.d.) were prepared by filling the pre-incubated sediments, which were mixed with different organic materials. About 7 g wet marine diatom (MD), 5 g land grass (LG), 4 g salt marsh plant (SMP) and their mixture (~1/3 of each material) were separately added to four beakers containing 100 g pre-incubated sediments. Land grass and salt marsh plant were cut into small pieces (~2×2 mm) before addition to the sediments. Materials and sediments were then mixed by hand stirring for 30 min. Addition of organic materials resulted in approximately 3-4% increase in TOC content in each treatment (Fig. 4.2). The plugs filled with the sediments were then incubated in the water reservoirs. Oxic and anoxic conditions were maintained by continuously purging the seawater with air or N_2/CO_2 mixture (the ratio of N_2 and CO_2 was chosen to keep a constant pH close to

8.1). The upper surface of the sediment plugs was exposed to well-stirred reservoirs, allowing for diffusive exchange of solutes between sediment and overlying water. The incubations were carried out in the dark at a stable temperature (14±1°C) over three months. At 0, 4, 13, 27, 45, 63, and 90 days, one plug of each treatment was withdrawn from reservoirs. The sediments were immediately stored at -40^{o} C for later analyses.

Bulk parameter analysis. Bulk parameters, including TOC, TN, $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TON}$, were determined using a Carlo Erba CHN analyzer coupled to a Finnigan Delta C Stable Isotope Mass Spectrometer. First, ~0.5 g thawed sediment samples were treated with 10% HCl solution (sonicating in a water bath for 5 min) to remove carbonate. After air-drying in a fume hood followed by freeze-drying, the samples were ground to fine powder for elemental and bulk isotope analyses. Additional ~0.5 g samples were dried at 60°C to measure water content of the sediments.

Extraction and analysis of fatty acids. Approximately 2 g thawed sediment samples were first extracted with 15 ml methanol, followed by 3×15 ml mixed solvents (methylene chloride/methanol, 2:1 in volume). During each extraction, samples were sonicated for 6 min and then centrifuged at 3000 rpm for 5 min to separate extract. The combined extracts were partitioned into a methylene chloride phase formed by the addition of 5% NaCl solution. After the volume was reduced using a rotary evaporator, the lipid extracts were saponified in 0.5 M KOH/MeOH. Neutral lipids were extracted with hexane out of the basic solution (pH > 13) while fatty acids were extracted after acidification to pH < 2. Fatty acids in the extracts were methylated with BF_3-methanol to form fatty acid methyl esters (FAMEs).

FAMEs were quantified by using a Hewlett-Packard 6890 GC with an on-column injector and a flame ionization detector. Compound separation was achieved by a 30 m × 0.25 mm i.d.

column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5, Hewlett-Packard). An internal standard (nonadecanoic acid methyl ester) was added to samples immediately prior to GC analysis to aid the quantification. The GC temperature program was: 50-150°C at 20°C min^{-1}, followed by 150-310°C at 4°C min^{-1}, and held at the final temperature for 5 min. Selected samples were analyzed by a GC-MS system (QP-5000, Shimadzu) and the structures of fatty acids were identified based on mass spectra. The GC-MS system was operated at 70 eV (ionizing energy) and the temperature program was the same as that used in the GC analysis.

Compound-specific isotope analysis. Stable carbon isotopic ratios of individual fatty acids were determined using a Varian 3400 GC-combustion system interfaced with a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS). The compounds were separated with a 30 m × 0.25 mm i.d. column (DB-1, J&W Scientific) and the GC temperature was programmed as: 50-150°C at 20°C min^{-1}, followed by 150-310°C at 4°C min^{-1}, and held at the final temperature for 5 min. Peaks eluting from the GC were combusted to CO_2 over CuO/Pt wires at 850°C and on-line transported to the IRMS. The isotopic compositions of CO_2 peaks were measured with the IRMS operated at 10 kV acceleration potential and by magnetic sector mass separation. The $\delta^{13}C$ ratios of the compounds were calibrated with a reference CO_2 gas ($\delta^{13}C$ = -11.23‰ relative to PDB). The standard deviation of IRMS was within ±0.56‰ based on internal standard measurements (n = 20). The $\delta^{13}C$ ratios of FAMEs were corrected for the extra carbon atoms added by methylation.

Kinetics model for degradation. In this study, we used a two-component kinetics model, which was adopted from the multi-G model (Berner, 1980), to estimate degradation rate constants of organic materials. It is assumed that there are two fractions, which have distinct reactivities in testing organic materials or even organic compounds (bound in different matrixes).

The overall degradation rate is equal to a sum of each individual degradation rate.

$$dC_t/dt = -(k_1C_1 + k_2C_2)$$

$$C_t = (C_1)_0 \exp(-k_1t) + (C_2)_0 \exp(-k_2t)$$

where C_t, C_1 *and* C_2 are the concentrations of total, fraction 1 (reactive) and fraction 2 (refractory); k_1 and k_2 are the first-order degradation rate constants for fractions 1 and 2; $(C_1)_0$ and $(C_2)_0$ are the initial (at t = 0) concentrations of fraction 1 and fraction 2. In some cases, k_2 is so small (close to zero) that the fraction 2 can be assumed to be non-degradable (C_{NR}). Thus,

$$C_t = (C_1)_0 \exp(-k_1t) + C_{NR}$$

In order to directly compare overall degradation rate constants no matter how the fractions vary under different conditions, we defined a new rate constant (k_{av}), which is an average degradation rate constant based on two fractions;

$$k_{av} = k_1 \times f_1 + k_2 \times f_2$$

where f_1 and f_2 are relative proportions of fraction 1 and fraction 2 [$f_1 = (C_1)_0/(C_t)_0$ and $f_2 = (C_2)_0/C_t)_0$]. The fraction sizes $(C_1)_0$ and $(C_2)_0$ were estimated by plotting ln (C_t) vs. incubation time (t), which resulted in a breaking point to differentiate two fractions.

Results

Variations of bulk parameters. Elemental and bulk isotopic parameters varied differently in various incubations (Fig. 2). TOC contents in the sediments (spiked with different organic materials) generally decreased during incubations but approached relatively constant levels at different times (one or two months, depending on the added materials). After three-month incubations, the lowest TOC remained in the sediments with addition of marine diatom while the highest TOC in the sediments with addition of salt marsh plants. TN contents in all sediment

treatments continuously declined with time but they were still higher that in the background sediments after three months. Although the initial C/N ratios in the spiked sediments were either higher or lower than that in the background sediments, they all varied toward the background ratio during incubations. Similarly, $\delta^{13}C$ and $\delta^{15}N$ ratios in different treatments also varied toward those in the background sediments. There seemed to be no remarkable differences in variations of all parameters between oxic and anoxic conditions. Moreover, bulk parameter variations in the sediments with addition of mixed materials were moderate, compared to those in the sediments with addition of single organic material.

Fatty acids compositions of sediments with different treatments. Fatty acid compositions of sediments with different treatments were initially different (Fig. 4.3). Since the concentration of fatty acids in the background sediments (pre-incubated over six months) was very low (Fig. 4.4 – 4.6), the fatty acid compositions in the treatment sediments actually represent those in the organic materials. For the sediment spiked with MD, 14:0, 16:1, and 16:0 were major (> 25% for each) components and polyunsaturated 20:4/20:5 fatty acids accounted for a significant (~10%) fraction. For the sediments spiked with LG and SMP, the fatty acid compositions were dominated by polyunsaturated 18:2/18:3 (50-60%) and 16:0 (20-30%). When the mixed organic materials were added to the sediments, the fatty acid composition was characterized by similar percentages of representative fatty acids from each organic material. Thus, we chose 14:0, 16:1, and 20:4/20:5 fatty acids as proxy of MD while 18:2/18:3 fatty acids as proxy of LG and SMP. The 16:0 fatty acid is non-specific and can be used to compare relative degradations of various materials among incubations. Although iso-15:0 and anteiso-15:0 fatty acids accounted for a very small proportion (< 1%) in the initial compositions, they are exclusively from bacteria and their variations (with their $\delta^{13}C$ compositions) during incubations can provide insights on bacterial

response and relative utilization of different organic materials.

Variations of source-specific fatty acids during incubations. Concentrations of MD-derived fatty acids (14:0, 16:1, and 20:4/20:5) decreased continuously during incubations of sediments spiked with MD and mixed materials (Fig. 4.4). In the first two weeks, 20:4/20:5 fatty acid concentrations dropped to almost zero while a small fraction (10-15% of initial concentration) of 14:0 and 16:1 fatty acids remained in the sediments and degraded at relatively slower rates until reaching the background concentrations. The differences in concentration variations between oxic and anoxic incubations seemed to be insignificant (for 20:4/20:5) or small (for 14:0 and 16:1), but there appeared to be some differences in concentration variations of 14:0 and 16:1 fatty acids between the sediment spiked with sole MD and the sediment spiked with mixed materials. Similar degradation patterns as MD-fatty acids were seen for 18:2/18:3 fatty acids (derived from LG and SMP) during incubations of sediments spiked with LG, SMP, and mixed materials (Fig. 4.5). The concentrations of these fatty acids dropped to zero within two weeks (in sediments spiked with LG or SMP separately) or in one month (in the sediment spiked with mixed materials). The differences between oxic and anoxic incubations were also insignificant (for LG and SMP addition cases) or small (for mixed material case).

For 16:0 fatty acid (non-specific, from all materials), the concentrations in all incubations did not declined to the background level over three months (Fig. 4.6). Apparently, 16:0 derived from MD degraded fastest compared to those derived from LG or SMP. On the other hand, 16:0 in the sediment spiked with mixed materials degraded at comparable rate as those in sediments spiked with LG and SMP.

Variations of bacteria-specific fatty acids during incubations. The concentrations of two typical bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) increased immediately and

reached the maximum levels in two weeks (Fig. 4.7). After two months, the concentrations gradually decreased and approached the background levels at the end of incubations. In the sediments spiked with LP, SMP, or mixed materials, the concentrations of bacteria-specific fatty acids seemed to be higher under anoxic conditions than under oxic conditions. However, in the sediments spiked with MD, there was little difference in the variations of bacteria-specific fatty acid concentrations between oxic and anoxic conditions.

Variations of compound-specific isotopic ratios during incubations. The $\delta^{13}C$ ratios of 16:0 fatty acid (non-specific, from all sources) differed distinctly in the sediments with different treatments at the beginning of incubations (t = 0) and varied differently during incubations (Fig. 4.8). The initial $\delta^{13}C$ ratio of 16:0 fatty acid in the sediments spiked with SMP was the most enriched (~ -24‰) while that in the sediments spiked with LG was the most depleted (~ -37‰). The initial $\delta^{13}C$ ratios of 16:0 fatty acids in the sediments spiked with MD and mixed materials were similar, in the almost middle between those in the sediments spiked with SMP and LG. In the first month of incubation (fast degradation period), the $\delta^{13}C$ ratios of 16:0 fatty acid in the sediments varied differently (depletion or enrichment) in a relatively larger range (up to ~ 6‰). After one month of incubation (slow degradation period), the $\delta^{13}C$ ratios of 16:0 fatty acid in the sediments spiked with individual LG, SMP, and MD varied in a relatively smaller range. However, at the end of incubations, the $\delta^{13}C$ ratios of 16:0 fatty acid in the sediments spiked with mixed materials were close to those in the sediments spiked with SMP.

At the beginning of incubations (t = 0), $\delta^{13}C$ ratios of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) in all sediments with different treatments were similar but varied differently during incubations (Fig. 4.9). In the sediments spiked with SMP, the $\delta^{13}C$ ratios of bacteria-specific fatty acids increased (enriched) in the first month (rapid degradation period). In

contrast, in the sediments spiked with LG, the $\delta^{13}C$ ratios decreased (depleted) in the first month. The $\delta^{13}C$ ratios of bacteria-specific fatty acids in the sediments spiked with MD and mixed materials varied less than those in the sediments spiked with SMP or LG in the first month. After one-month incubation (slow degradation period), the $\delta^{13}C$ ratios of these bacteria-specific fatty acids varied in smaller ranges compared to those in the first month. However, the $\delta^{13}C$ ratios were distinctly different among three sediments spiked with SMP, LG, and MD. The $\delta^{13}C$ ratios in the sediments spiked with mixed materials were close to those in the sediments spiked with MD.

Discussion

Biochemical reactivities of different organic materials. Several different degradation patterns were observed in this study. For total organic carbon (TOC), degradation occurred in the first one or two month of incubations and afterward a non-reactive fraction was always present in all sediments with different treatments. In contrast, polyunsaturated fatty acids (20:4/20:5 and 18:2/18:3), regardless of their sources, degraded completely within the first month of incubations. For other MD-derived fatty acids (e.g., 14:0 and 16:1), degradation took place at two distinct stages: rapid decay in the first month followed by a slow degradation until reaching the background levels. Non-specific fatty acid 16:0 (from all sources) also showed a two-stage degradation but there was a fraction that remained in the sediments even after three months. Diversified degradation patterns implied that degradation processes of organic matter are affected not only by the compositions (source related) but also by the compound structures.

Harvey et al. (1995) observed that proteins and carbohydrates of algal cells (diatom *T. weissflogii* and cyanobacterium *Synechococcus sp*.) were completely degraded while lipids and

POC were partially degraded within 150 days of incubations. Other studies (Maccubbin and Hodson, 1980; Wilson, 1985; Benner et al., 1986) have demonstrated that the carbohydrates of vascular plants are degraded much faster than the lignin component, resulting in an enrichment of lignin-derived carbon in the detritus. It appears that the non-reactive fractions in our experiments (higher % in SMP than in MD) consist of recalcitrant macromolecules such as lignin, tannin, suberin and cutin in higher plants (De Leeuw and Largeau, 1993) and some uncharacterized refractory components in marine phytoplankton (Wakeham et al., 1997).

On the other hand, structural unsaturation plays an important control for degradation of many lipid compounds (Harvey and Macko, 1997a). The abundances of saturated fatty acids are widely observed to increase in sediments relative to their source materials while the abundances of unsaturated fatty acid are generally decreased (Farrington et al., 1977; Wakeham et al., 1991; Haddad et al., 1992). Especially, polyunsaturated fatty acids are highly susceptible to microbial degradation and assimilation by animals (Bradshaw and Eglinton, 1993). Thus, complete degradation of polyunsaturated fatty acids observed in this study is not affected by the material sources, instead, caused by the structural feature. Two degradation stages with distinct rates found for other fatty acids (14:0, 16:1, and 16:0) may be partly related to molecular associations (complexes) within material matrixes. It is well known that plant fatty acids are mostly bound in triacylglycerols (intracellular storage component) and phospholipids (cell membrane). Ding and Sun (2005) reported that fatty acids bound in membrane and intracellular components degraded at different rates, probably due to their different susceptibilities to degrading enzymes.

Since degradation processes of different organic materials involve different chemical components and their relative pool sizes vary from material to material, the overall reactivities among different materials are best compared based on average degradation rate constants, which

are derived from all reactive and refractory pools. The comparison of the average rate constants of TOC (Table 4.1) revealed that under both oxic and anoxic conditions, the overall reactivities of organic materials varied by the following order: marine diatom > land grass > salt marsh plant. This order is consistent with the general view for the reactivities of marine phytoplankton and terrestrial higher plants (Ittekkot and Lanne, 1991).

A comparison of degradation rate constants of individual compounds also provided an insight on the relative reactivities of different organic compounds in our experimental sediments. For example, 16:0 fatty acid (non-specific) was present in all materials but degraded at different rates in various incubations (Table 4.2). The highest rate constants (k_{av}, under both oxic and anoxic conditions) of 16:0 fatty acid degradation were found in the sediments spiked with MD while the lowest rate constants in the sediments spiked with SMP. For 18:2/18:3 fatty acids, which were found in SMP and LG rather in MD, the degradation rate constants (k_{av}) were higher in the sediments spiked with LG than those in the sediments spiked with SMP (Table 4.3). Consistent reactivity orders of TOC and individual compounds confirm that the relative proportions of organic matter from different end-member sources are an important factor affecting overall degradation of OM in estuarine sediments.

Variations in $\delta^{13}C$ ratios of 16:0 fatty acid during the incubations further confirmed the relative reactivities of different organic materials in our experimental systems (Fig. 4.8). In the sediments spiked with single organic material, the initial $\delta^{13}C$ ratios of 16:0 fatty acid bound in different organic materials were distinct, ranging from ~ -24‰ (for SMP) to ~ -37‰ (for LG). Although there were larger shifts in $\delta^{13}C$ of 16:0 fatty acid during the first month than in the later incubations, the $\delta^{13}C$ ratios in each sediment (with different organic material added) were well separated. The reason for larger shifts in molecular $\delta^{13}C$ in the first month of incubations is at

least partly due to the isotopic fractionation during remarkable degradation (Meckenstock et al., 1999; Sun et al., 2004; Dai et al., 2005). In the sediment spiked with mixed organic materials, however, the $\delta^{13}C$ ratios of 16:0 fatty acid gradually approached to those of SMP during the incubations, implying that the remaining 16:0 fatty acid in the sediments spiked with mixed materials at the end of incubations is dominated by those from SMP. This conclusion is consistent with the order in the degradation rate constants (Table 4.2), that is, SMP might be least degraded compared to MD and LG.

Effects of redox conditions on organic matter degradation. Although in all cases, including TOC and individual fatty acids, oxic degradation seemed to be faster than anoxic degradation, the redox effects were not obvious. The ratios of k_{ox}/k_{an} are generally less than 2 (with an exception for 16:0 fatty acid in the sediments with addition of SMP). Many previous studies (Harvey et al., 1995; Harvey and Macko, 1997a; Sun et al., 1997, 2002) reported that algal lipid compounds degraded much faster under oxic conditions than under anoxic conditions with k_{ox}/k_{an} ratios greater than 3. The less redox effects on degradation observed in this study may be related to variations of microbial communities caused by 6-month pre-incubations of experimental sediments.

In previous studies, incubation systems were setup by using intact oxic and anoxic seawater or sediment containing natural aerobic and anaerobic bacteria or by introducing viable anaerobic microbial consortia. In this study, in order to reduce the content of labile organic matter in the experimental sediments, we pre-incubated the sediments over six months. Indeed, labile components were mostly removed during the pre-incubation, indicated by an absence of unsaturated fatty acids. However, the bacteria abundance might be greatly reduced, indicated by a very low concentration of bacteria-specific fatty acids in the sediments at t = 0. It is unclear

how the microbial community is altered during the pre-incubation. Probably, only some highly resistant bacteria species can survive. When the sediments (with addition of different organic materials) were exposed to oxic and anoxic seawaters, the survived bacteria might act facultatively. They degraded the added organic materials at similar efficiency, leading to a less effect of redox conditions on organic matter degradation.

Effects of co-metabolism on organic matter degradation. Estuarine and coastal marine sediments receive refractory organic matter from land but only a small fraction of the terrestrial organic matter is preserved in the marine sediments (Hedges et al., 1997). Degradation of the more refractory land-derived organic matter in the sediments is thought to be caused by a linkage to the decay of the labile organic matter produced in the ocean (Wakeham and Canuel, 2006). This co-metabolism relationship implies that a large amount of refractory organic matter decomposition could potentially occur in the upper metabolically-activity zone of the sediments (Smith et al., 1992; Canfield, 1994).

In this study, however, when various organic materials (with almost equal proportions) were together incubated in the sediments, the co-metabolism effect appeared to be insignificant. For TOC, the k_{av} (average degradation rate constant) in the sediments with mixed materials is close to that in the sediments spiked with only SMP but far lower than those in the sediments spiked with either LG or MD (Table 4.1). Based on the individual k_{av} from incubations of sediments spiked with single material and the relative proportions of each material in the sediments with mixed materials, we calculated an average rate constant $(k_{cal})_{mix}$ for overall degradation of TOC in the sediments with mixed materials. In fact, the calculated rate constants are more than 3× higher than those derived from actual measurements (Table 4.5). This comparison clearly indicates that presence of reactive MD did not stimulate the degradation of

refractory SMP in the sediments. Similar patterns were observed for non-specific 16:0 fatty acid (Tables 4.2 and 4.5).

Although the effect of co-metabolism (or priming) on organic matter degradation was discovered by soil scientists a long time ago (Löhnis, 1926), the causes and mechanisms have not been well understood (Kuzyakov et al., 2000). The positive effect, which is to accelerate soil organic matter degradation with addition of labile organic substrate, is attributed primarily to increase in microorganism activity (Azam et al., 1994; Lavelle and Gilot, 1994). The negative effect, which is to retard organic matter degradation, has also been observed (Sparling et al., 1982; Cheng, 1996, 1999; Wang and Bakken, 1997). The major causes for negative effect are probably due to switch of organic matter sources for bacteria, decrease in C to N ratio, sorption or physical-chemical protection, and C-immobilization in bacterial biomass. Canfield (1994) proposed a pseudo-G model to establish relationships between labile and refractory organic fractions, but the co-metabolism effects have been tested to vary in different biogeochemical regimes (e.g., with or without bioturbation). In this study, an absence or negative co-metabolism effect on degradation of various materials in the aged sediment was observed but it is unclear what the cause is. Changes in microbial community over a six-month pre-incubation may be responsible for this negative effect. Although concentrations of bacteria-specific fatty acids varied markedly and similarly in all treatments (additions of single material or mixed materials), the functions of bacteria and the interactions with different organic materials in different treatments are unknown.

Bacterial response to different organic materials. Bacteria play dual roles in organic matter cycling: (1) as primary agents to degrade dissolved and particulate organic matter, and (2) as producers for new particulate organic matter by transforming dissolved substrates into cellular

biomass (Azam et al., 1983; Deming and Baross, 1993). On the other hand, bacterial biomass is subject to consumption by microeucaryotes or biochemical degradation (Harvey and Macko, 1997b).

During growth, bacteria can biosynthesize a series of specific compounds such as odd number C_{15}-C_{17} branched fatty acids (Kaneda, 1991), which have been used as bacterial source indicators (Parkes and Taylor, 1983; Cranwell et al., 1987; Wakeham et al., 1991). Variations of bacteria-specific fatty acids (iso- and anteiso-15:0) during our incubations provide an insight on bacterial response to addition of organic materials into the sediments. Regardless of materials, the concentrations of bacteria-specific fatty acids in all sediments with different treatments increased rapidly in the first week and maintained high concentrations within almost two months, suggesting that bacterial growth was stimulated by addition of any fresh organic materials. The stimulation of fresh organic matter on growth of bacteria has been observed in other studies (Danovaro et al., 1994). After two months of incubations when most reactive organic matter was degraded, the concentrations of bacteria-specific fatty acids gradually decrease toward the initial levels in the background sediments, implying that newly-produced bacterial biomass was turned over. Similar variation patterns of bacteria-specific fatty acids were observed in a previous study (Ding and Sun, 2005). However, in that study, the variations of bacteria-specific fatty acid concentrations did not correlate with those of bacterial abundance and related enzyme activities. As pointed out by Harvey and Macko (1997b), the relationship between bacterial biomass and bacteria-specific fatty acids is complicated, which may be affected by several opposite processes such as production vs. turnover of biomass and biosynthesis vs. degradation of specific compounds.

A number of studies (Canuel et al., 1997; Boschker et al., 1999; 2005) have demonstrated

that $\delta^{13}C$ ratios of bacteria-specific fatty acids can be used to assess the sources of organic matter utilized by bacteria in estuarine and coastal environments where organic materials come from different sources. In this study, we added three end-member organic materials with distinct $\delta^{13}C$ ratios into the experimental sediments separately or together. Initially (t = 0), the $\delta^{13}C$ ratios of two bacteria-specific fatty acids in all experimental sediments were the same but they varied differently in sediments with addition of different organic materials during incubations. In the sediments spiked with LG, the $\delta^{13}C$ ratios became depleted relative to the initial vales, reflecting a use of C3 terrestrial organic carbon by bacteria while in the sediments spiked with SMP, the $\delta^{13}C$ ratios became enriched, indicating a use of C4 salt marsh plant-derived organic carbon. Our previous study (Dai et al., 2005) also showed that the $\delta^{13}C$ ratios of bacteria-specific fatty acids produced during incubations were material-dependent. The $\delta^{13}C$ ratios of bacteria-specific fatty acids in sediments spiked with only MD and mixed materials varied in a smaller range relative to those in sediments spiked either with LG or with SMP. Interestingly, those variations in the sediments spiked with MD and mixed materials were closely similar, implying that even though a variety of organic materials are present in the sediments, bacteria indeed utilize the most reactive organic carbon from MD.

Conclusions

This study determined the chemical and isotopic variations of bulk parameters and individual fatty acids in the sediments with additions of different organic materials and variable redox conditions. The kinetic modeling results revealed that the relative bioreactivities of the major organic materials in Altamaha estuarine sediments were in the following sequence: marine diatom > land grass > salt marsh plant. It was found that redox conditions had a small effect on

degradation of fresh materials, regardless of the sources, in the aged sediments. When labile marine diatom and refractory land grass and salt marsh plant were together incubated in the sediments, no effect of co-metabolism was observed. Variations in concentration of bacteria-specific fatty acids during incubations indicated that bacterial community had a rapid response to any fresh organic material whenever it was the only input to the sediments. Based on the variations in $\delta^{13}C$ of bacteria-specific fatty acids, however, it is clear that bacteria preferentially utilize relatively labile organic carbon from marine diatom over those bound in land grass or salt marsh plant when all materials co-exist in the sediments.

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Table 4.1. Degradation rate constants (k, day^{-1}) of TOC in sediments with addition of different organic materials.

	k_1	k_2	f_1	f_2	k_{av}	(r^2)	k_{ox}/k_{an}
TOC, oxic							
LG	0.258	0.000	0.610	0.390	0.157	(0.585)	**1.355**
SMP	0.071	0.000	0.519	0.481	0.037	(0.784)	**1.638**
MD	0.310	0.000	0.805	0.195	0.249	(0.952)	**1.131**
Mixed	0.067	0.000	0.696	0.304	0.047	(0.831)	**1.458**
$(k_{av})_{LG}/(k_{av})_{mixed}$					*3.372*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*0.790*		
$(k_{av})_{MD}/(k_{av})_{mixed}$					*5.347*		
TOC, anoxic							
LG	0.208	0.000	0.558	0.442	0.116	(0.669)	
SMP	0.051	0.000	0.441	0.559	0.022	(0.825)	
MD	0.318	0.000	0.693	0.307	0.221	(0.962)	
Mixed	0.064	0.000	0.500	0.500	0.032	(0.898)	
$(k_{av})_{LG}/(k_{av})_{mixed}$					*3.628*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*0.703*		
$(k_{av})_{MD}/(k_{av})_{mixed}$					*6.892*		

Table 4.2. Degradation rate constants (k, day^{-1}) of non-specific (from all sources) 16:0 fatty acid in sediments with addition of different organic materials.

	k_1	k_2	f_1	f_2	k_{av}	(r^2)	k_{ox}/k_{an}
16:0, oxic							
LG	0.192	0.001	0.809	0.191	0.156	(0.872)	**1.667**
SMP	0.157	0.0075	0.610	0.390	0.099	(0.942)	**2.270**
MD	0.594	0.013	0.780	0.220	0.466	(0.989)	**1.639**
Mixed	0.188	0.016	0.536	0.464	0.108	(0.930)	**1.452**
$(k_{av})_{LG}/(k_{av})_{mixed}$					*1.439*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*0.913*		
$(k_{av})_{MD}/(k_{av})_{mixed}$					*4.313*		
16:0, anoxic							
LG	0.125	0.004	0.737	0.263	0.093	(0.997)	
SMP	0.069	0.007	0.591	0.409	0.044	(0.953)	
MD	0.359	0.006	0.789	0.211	0.284	(0.995)	
Mixed	0.146	0.016	0.450	0.550	0.074	(0.925)	
$(k_{av})_{LG}/(k_{av})_{mixed}$					*1.253*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*0.584*		
$(k_{av})_{MD}/(k_{av})_{mixed}$					*3.821*		

Table 4.3. Degradation rate constants (k, day^{-1}) of LG- and SMP- derived fatty acids in sediments with addition of different organic materials.

	k_1	k_2	f_1	f_2	k_{av}	(r^2)	k_{ox}/k_{an}
18:2/18:3, oxic							
LG	0.464	0.000	1.000	0.000	0.464	(0.999)	**1.234**
SMP	0.389	0.000	1.000	0.000	0.389	(0.991)	**1.131**
mixed	0.201	0.000	1.000	0.000	0.201	(0.993)	**1.661**
$(k_{av})_{LG}/(k_{av})_{mixed}$					*2.308*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*1.935*		
18:2/18:3, anoxic							
LG	0.376	0.000	1.000	0.000	0.376	(0.999)	
SMP	0.344	0.000	1.000	0.000	0.344	(0.993)	
mixed	0.121	0.000	1.000	0.000	0.121	(0.989)	
$(k_{av})_{LG}/(k_{av})_{mixed}$					*3.107*		
$(k_{av})_{SMP}/(k_{av})_{mixed}$					*2.843*		

Table 4.4. Degradation rate constants (k, day^{-1}) of MD-derived fatty acids in sediments spiked with MD and mixed organic materials.

	k_1	k_2	f_1	f_2	k_{av}	(r^2)	k_{ox}/k_{an}
14:0, oxic							
MD	0.640	0.018	0.947	0.053	0.607	(0.997)	**1.649**
mixed	0.384	0.028	0.908	0.092	0.351	(0.990)	**1.723**
$(k_{av})_{MD}/(k_{av})_{mixed}$					*1.729*		
14:0, anoxic							
MD	0.421	0.025	0.867	0.133	0.368	(0.999)	
mixed	0.217	0.0121	0.936	0.064	0.204	(0.981)	
$(k_{av})_{MD}/(k_{av})_{mixed}$					*1.807*		
16:1(n-7) , oxic							
MD	0.883	0.028	0.861	0.139	0.764	(0.998)	**1.448**
mixed	0.246	0.017	0.860	0.140	0.214	(0.995)	**1.332**
$(k_{av})_{MD}/(k_{av})_{mixed}$					*3.570*		
16:1 (n-7), anoxic							
MD	0.618	0.022	0.848	0.152	0.527	(0.999)	
mixed	0.187	0.0115	0.850	0.150	0.161	(0.979)	
$(k_{av})_{MD}/(k_{av})_{mixed}$					*3.282*		
20:4/20:5, oxic							
MD	1.121	0.000	1.000	0.000	1.121	(0.999)	**1.483**
mixed	0.731	0.000	1.000	0.000	0.731	(0.998)	**1.507**
$(k_{av})_{MD}/(k_{av})_{mixed}$					*1.534*		
20:4/20:5, anoxic							
MD	0.756	0.000	1.000	0.000	0.756	(0.999)	
mixed	0.485	0.000	1.000	0.000	0.485	(0.999)	
$(k_{av})_{MD}/(k_{av})_{mixed}$					*1.559*		

Table 4.5. Comparison of measured to calculated degradation rate constants (k, day^{-1}) of TOC and non-specific 16:0 fatty acid in sediments with addition of mixed organic materials.

	TOC		16:0	
	oxic	anoxic	oxic	anoxic
$(k_{av})_{mix}$	0.047	0.032	0.108	0.074
$(k_{cal})_{mix}$*	0.148	0.120	0.240	0.140
$(k_{av})_{mix}/(k_{cal})_{mix}$	**0.318**	**0.267**	**0.450**	**0.529**

*$(k_{cal})_{mix} = 1/3(k_{av})_{LG} + 1/3(k_{av})_{SMP} + 1/3(k_{av})_{MD}$

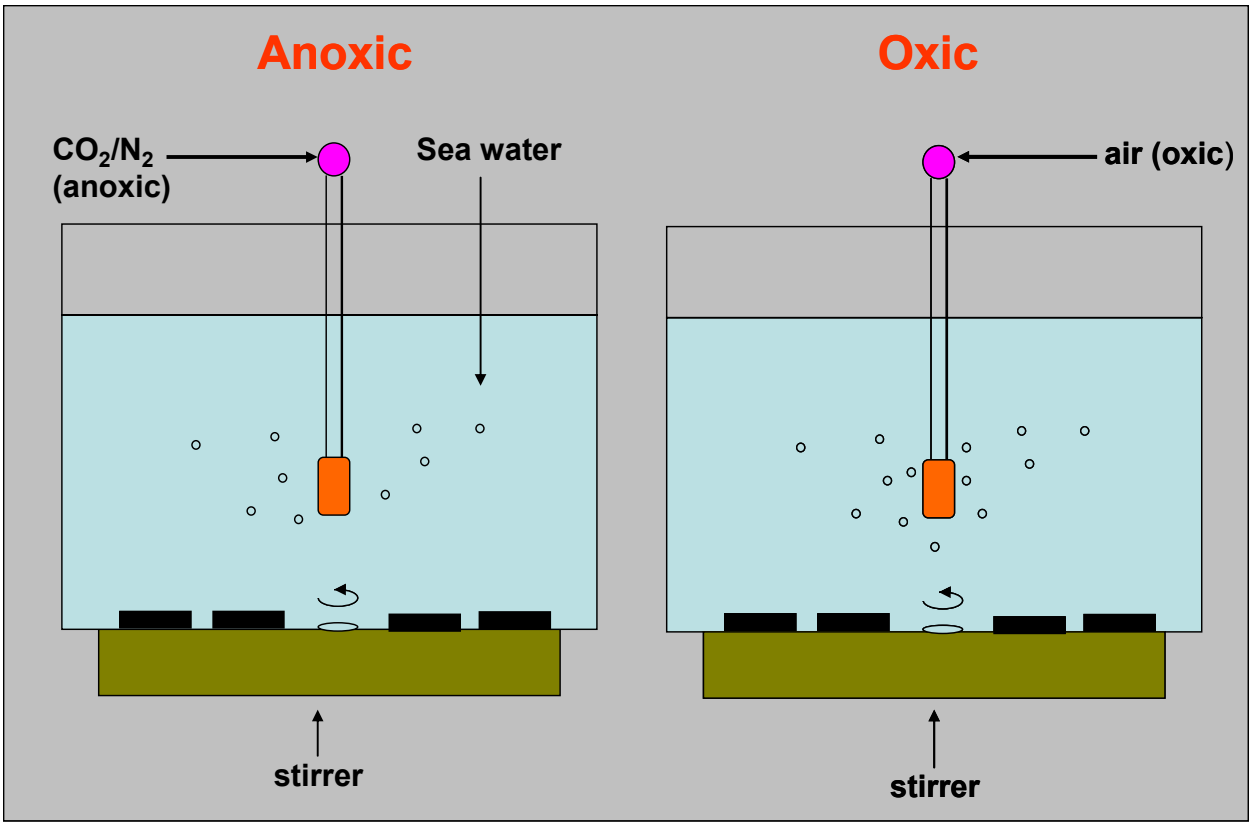

Fig. 4.1. Experimental incubation systems: sediment samples in plugs sitting on the bottom of water reservoirs (10 L) with purging of air or N_2/CO_2 gases.

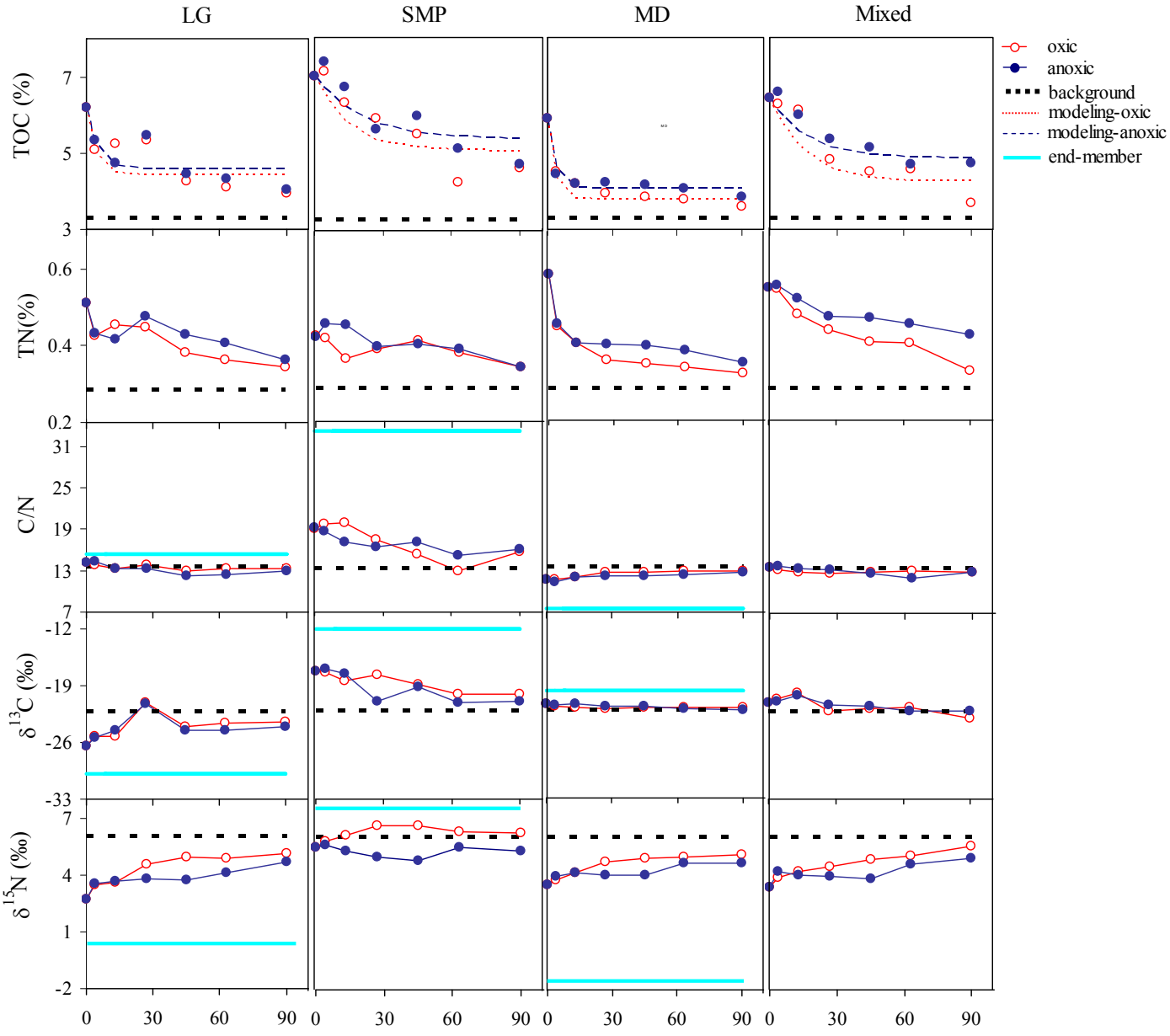

Fig. 4.2. Variations of bulk parameters (TOC, TN, C/N ratio, $\delta^{13}C$, and $\delta^{15}N$) in sediments with addition of different organic materials. The C/N ratios, $\delta^{13}C$, and $\delta^{15}N$ of three end-member organic materials (LG, SMP, and MD) are presented as solid straight lines.

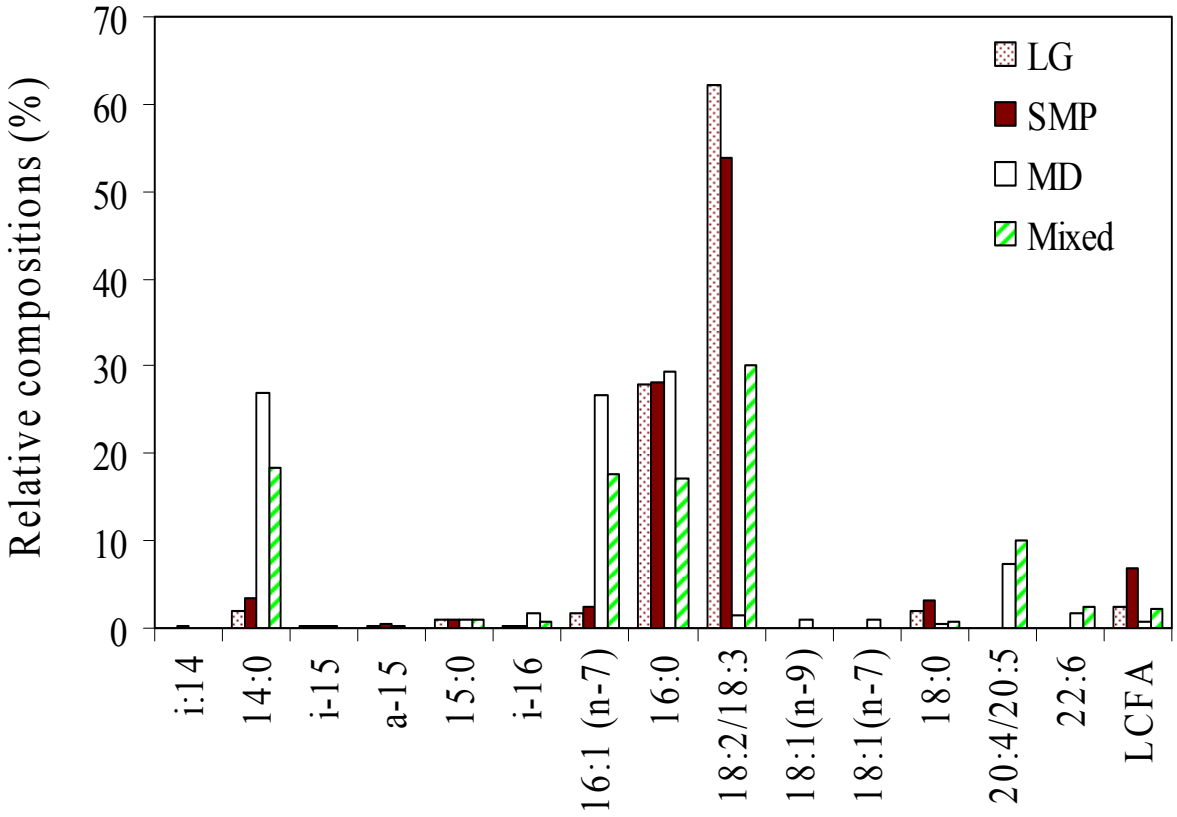

Fig. 4.3. Relative compositions of fatty acids in sediments with addition of different organic materials at the beginning of incubations (t = 0).

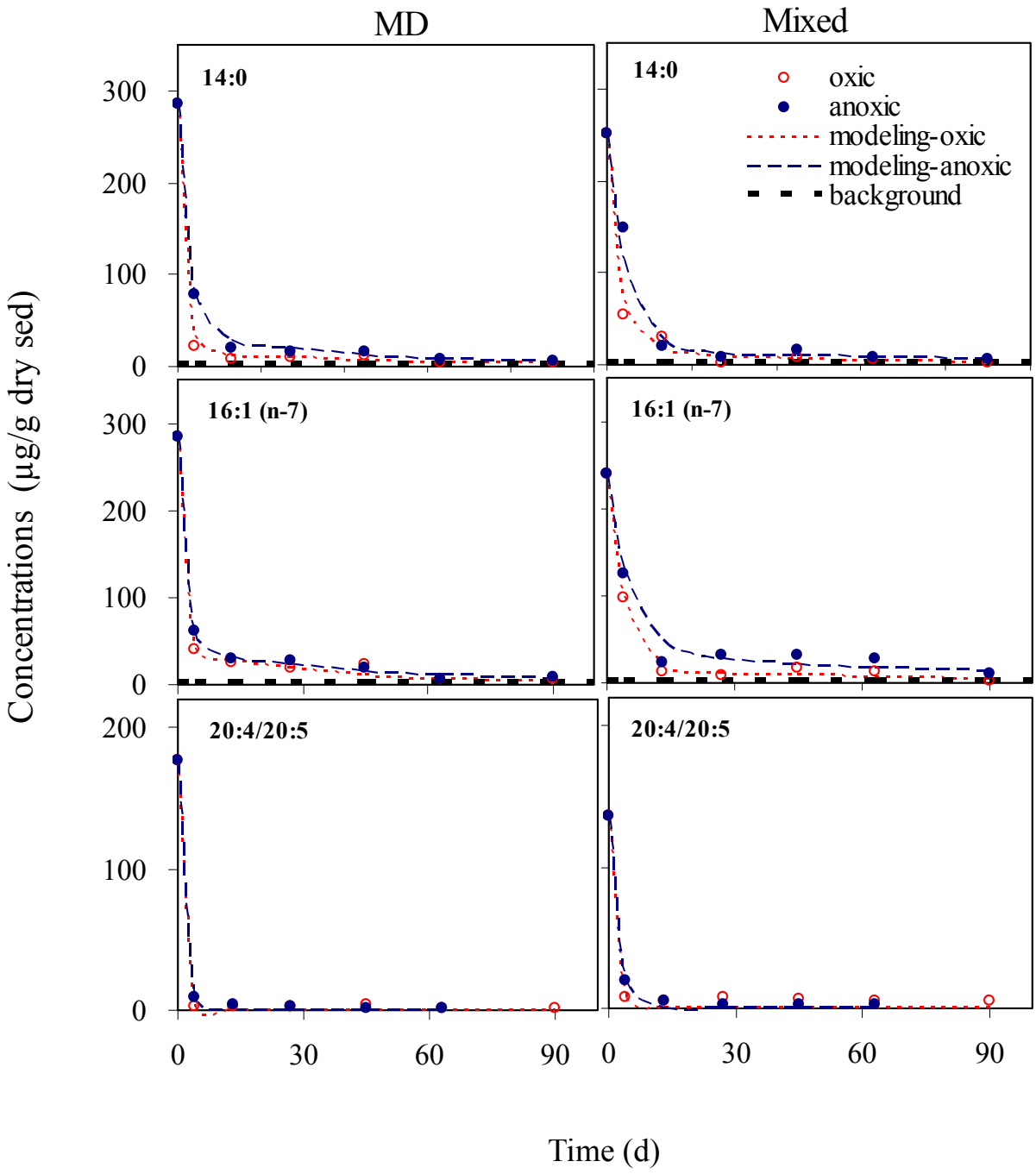

Fig. 4.4. Variations of MD-derived fatty acid concentrations during oxic and anoxic incubations. Dotted (oxic) and dashed (anoxic) lines are fits of a kinetic model.

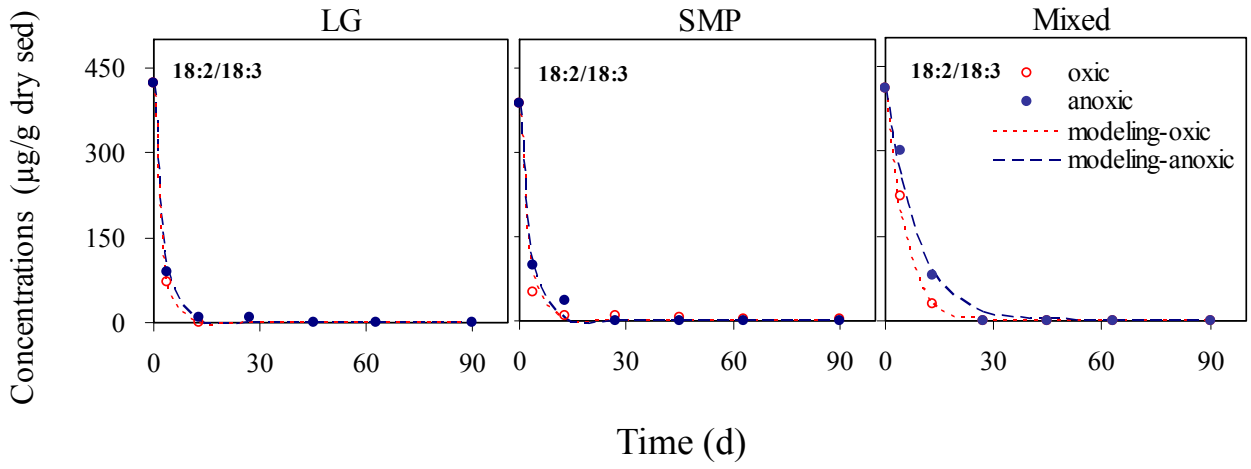

Fig. 4.5. Variations of LG- and SMP-derived fatty acid concentrations during oxic and anoxic incubations.

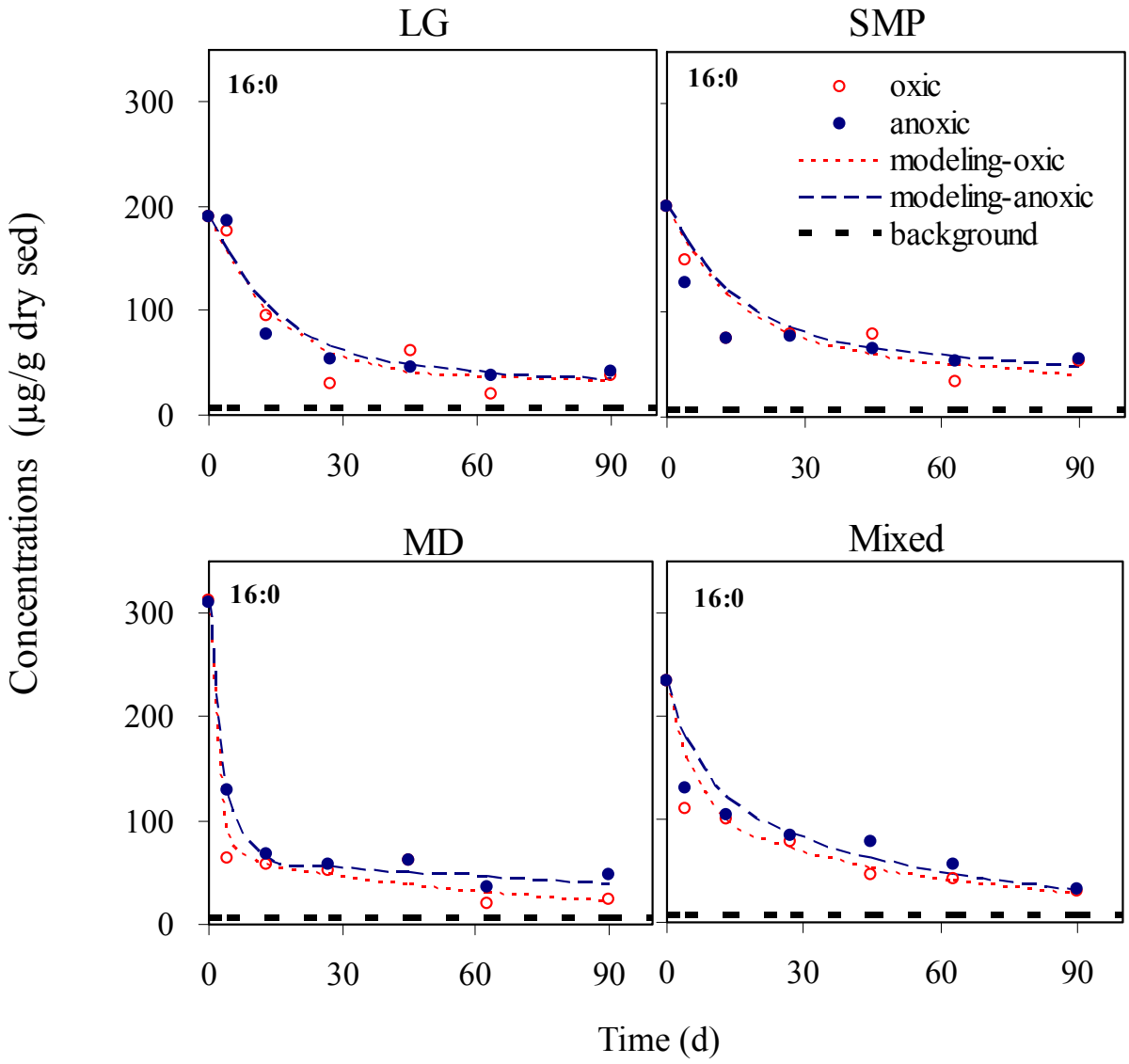

Fig. 4.6. Variations of non-specific (from all sources) 16:0 fatty acid concentrations during oxic and anoxic incubations.

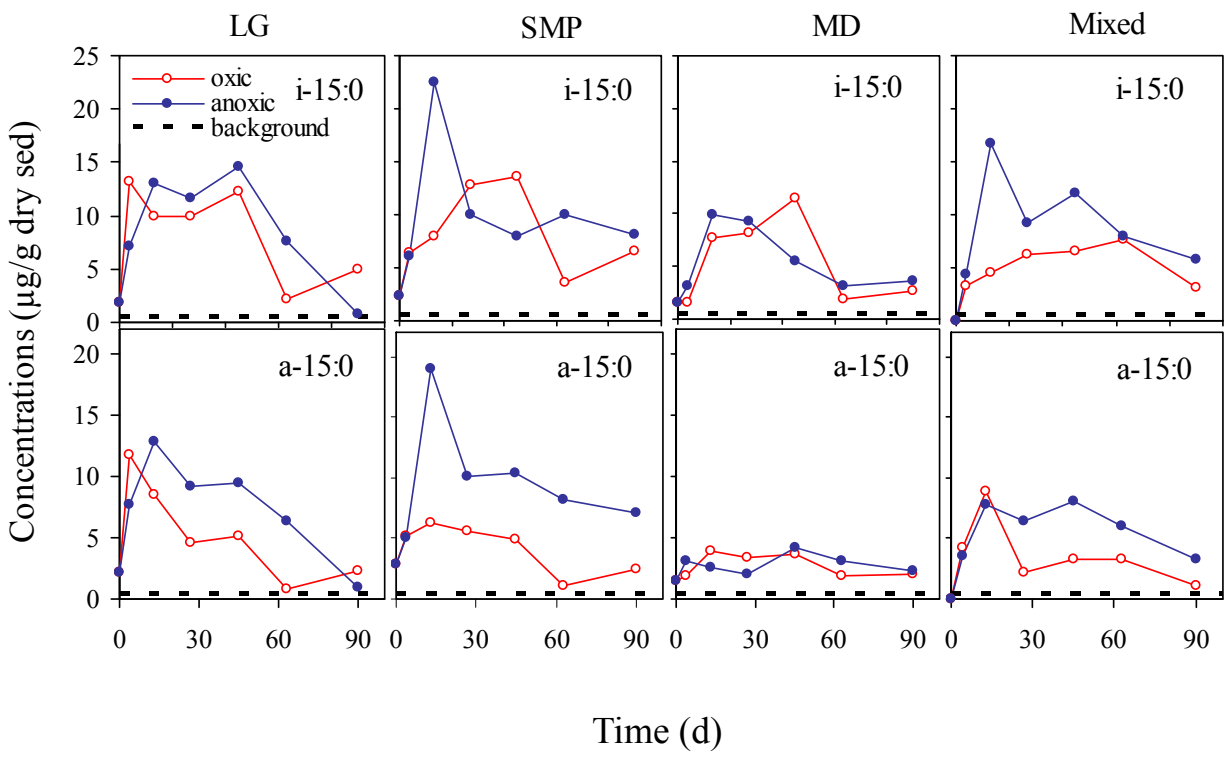

Fig. 4.7. Variations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) during oxic and anoxic incubations.

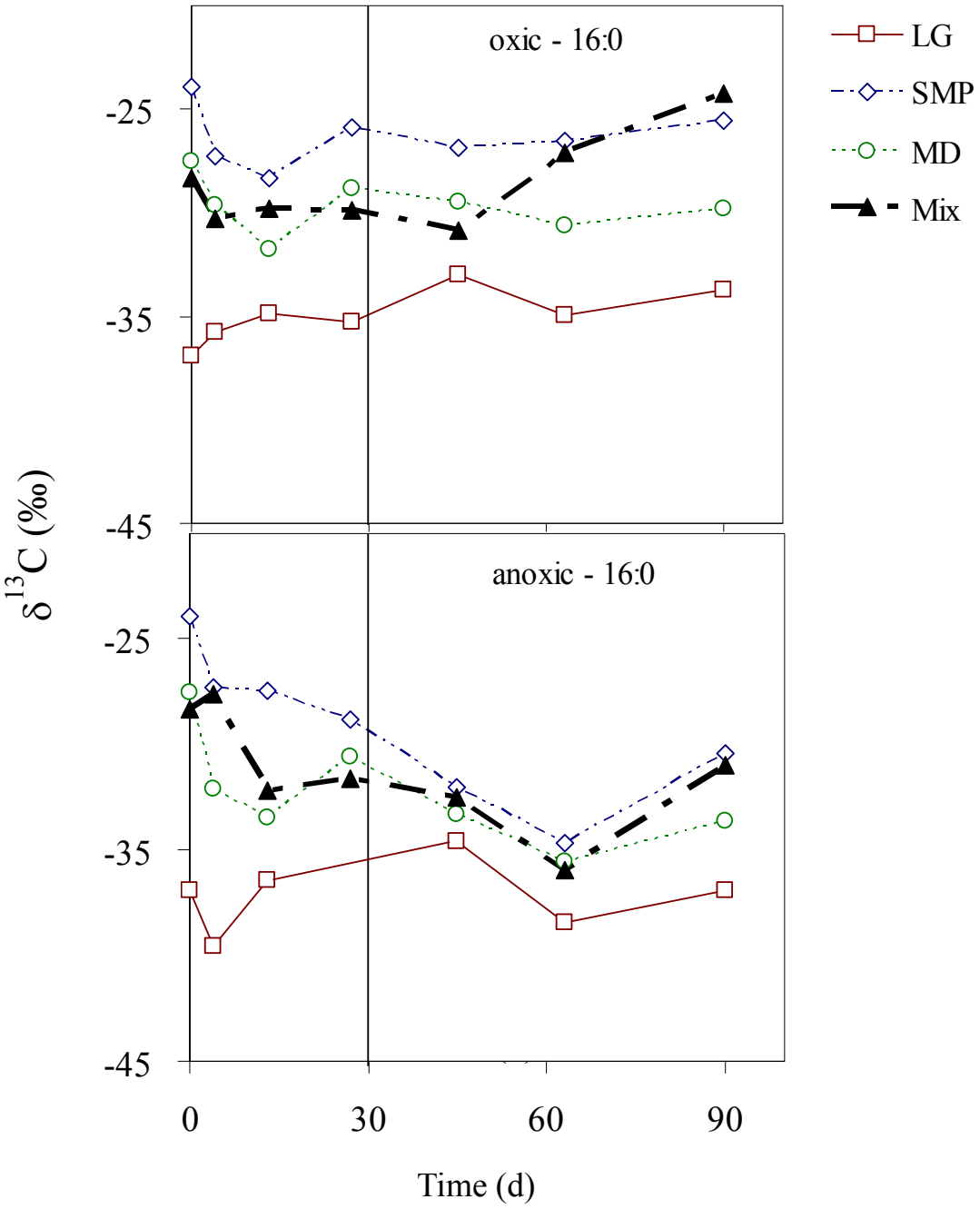

Fig. 4.8. Variations of $\delta^{13}C$ of non-specific fatty acid 16:0 during oxic and anoxic incubations. The vertical straight lines at 30 days represent two degradation stages.

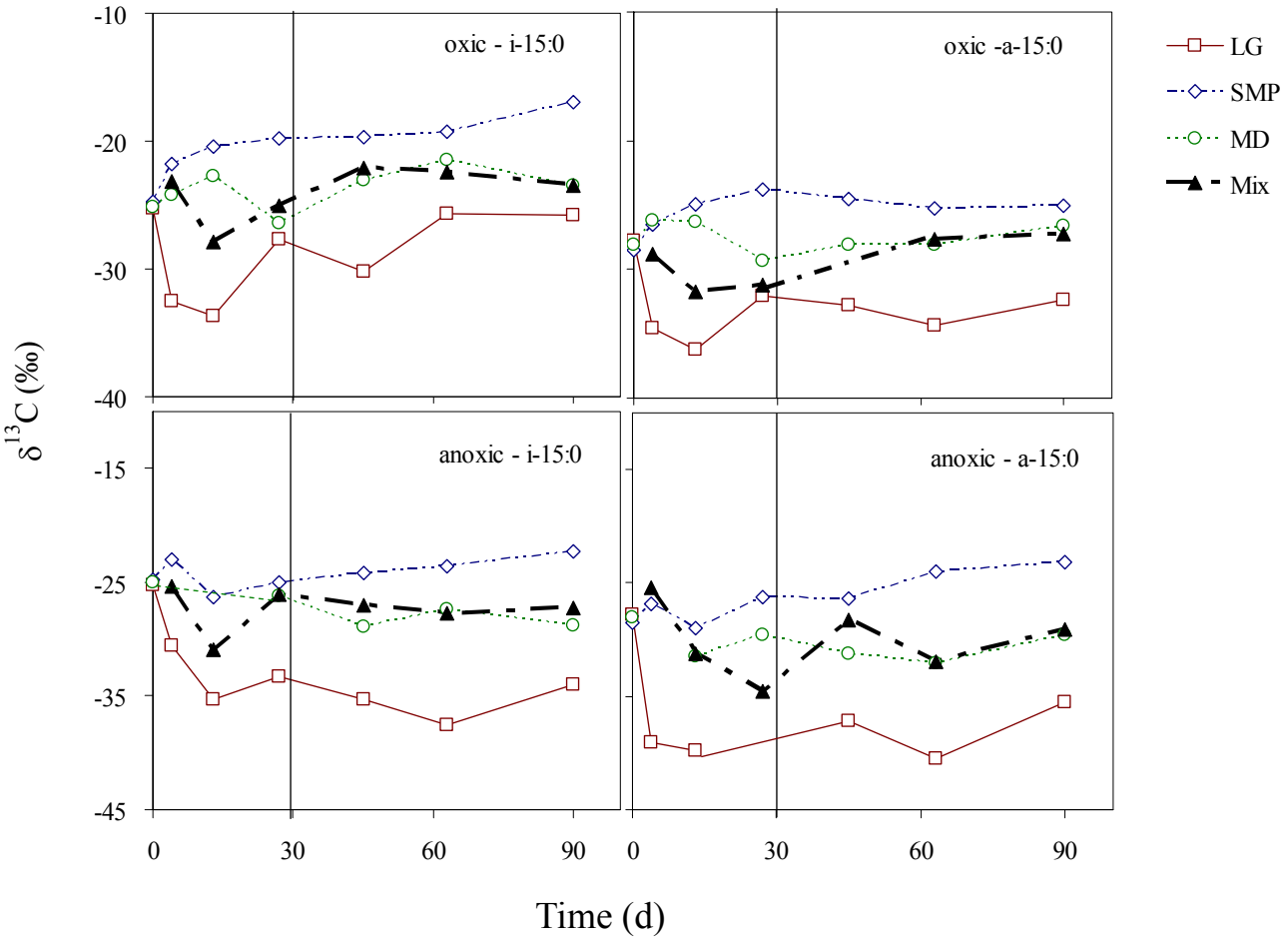

Fig. 4.9. Variations of $\delta^{13}C$ of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) during oxic and anoxic incubations. The vertical straight lines at 30 days represent two degradation stages.

CHAPTER 5

EFFECT OF CO-METABOLISM OF FRESH AND AGED ORGANIC MATERIALS ON ORGANIC MATTER DEGRADATION IN ALTAMAHA ESTUARINE WATER

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Abstract

This study examined the effect of co-metabolism of land-derived organic materials and marine phytoplankton on the overall degradability of organic matter in Altamaha estuarine water. Fresh and aged land grass (*Festuca arundinacea*) and salt marsh plant (*Spartina alterniflora*) and fresh marine diatom (*Skeletonema costatum*), which represent major organic matter inputs in the Altamaha River estuary, were mixed in different modes and incubated in the estuarine water over one month. TOC and $\delta^{13}C_{TOC}$, fatty acids and their $\delta^{13}C$ ratios were measured and a two-component degradation model was applied to determine the degradation rate constants of biomarkers derived from different sources. During the incubation, $\delta^{13}C_{TOC}$ in different treatments became enriched toward that of salt marsh plant, suggesting that salt marsh plant was least degraded. Land material-derived 18:2/18:3 fatty acids degraded significantly faster (~4×) in the treatment with addition of fresh marine diatom than in the treatment without marine diatom, indicating that the co-metabolism might stimulate the degradation of terrestrial organic matter. On the other hand, degradation rate constants of 14:0 and 16:1 fatty acids (primarily produced by marine diatom) in the treatment containing aged land grass and salt marsh plant were 2× lower than those in the treatment containing fresh land grass and salt marsh plant, implying that the presence of aged terrestrial organic matter might have a negative influence on degradation of marine organic matter. Concentrations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) increased apparently in the treatments containing all materials but varied in a smaller range in the treatments containing only land grass and salt marsh plant (fresh and aged), implying that the presence of marine diatom might enhance bacterial metabolism. In addition, the variations in $\delta^{13}C$ ratio of bacterial iso-15:0 fatty acid in different treatments further confirmed that bacteria preferentially utilized the carbon from marine diatom over that from terrestrial organic matter

when a variety of organic materials coexist in the system.

Keywords: Allochthonous and autochthonous organic matter, Source-specific biomarkers, Stable carbon isotopic ratios, Co-metabolism, Organic matter degradation, Bacterial response

Introduction

Estuaries play a critical role in global carbon cycling, serving as an interface for organic carbon transport from land to the ocean on one side and as a trap (or a sink) for organic carbon on the other side (Ittekkot, 1988; Mulholland and Olsen, 1992). Over decades, many studies (Darnell, 1967; Odum, 1968; Haines, 1976; Fogel et al., 1989; Hedges et al., 1997) have focused on the fate of organic matter and its significance to the food webs in estuarine systems. However, these issues are still not well resolved (Hopkinson et al., 1998). One key uncertainty is incomplete understanding on relative reactivities of organic materials from different sources, which directly control the overall degradation of organic matter in estuarine systems.

Although allochthonous (mostly derived from land) organic matter is generally considered to be refractory relative to autochthonous (aquatic production), increasing evidence showed that the both are important to drive biochemical cycling in many estuarine systems (Hopkinson and Vallino, 1995; Smith and Hollibaugh, 1995). Many studies have demonstrated that terrestrial organic matter is rapidly degraded in estuarine and coastal environments and only a small fraction of it is preserved in the marine sediments (Hedges et al., 1988a, b; 1997). This rapid recycling of terrestrial organic matter is, at least partly, attributed to the effect of co-metabolism of labile and refractory organic matter (Wakeham and Canuel, 2006). Co-metabolism is a process to stimulate the degradation of refractory organic matter by high metabolic activity resulted from

decay of labile organic matter (Smith et al., 1992; Canfield and van Cappellen, 1992). Canfield (1994) proposed a pseudo-G model to explore the relationships between degradation processes of labile and refractory organic matter. This hypothetical model predicts that decomposition of refractory organic matter is linked to decay of labile organic matter through different ways (linear, fractional, and squared) and these relationships may affect organic carbon preservation in the sediments with varying biogeochemical conditions (e.g., with and without bioturbation). Unfortunately, few studies have been conducted to test this theory in the estuarine systems.

On the other hand, diagenetic status (age) of organic matter has been considered as another intrinsic factor controlling the degradation processes. The study conducted by Canuel and Martens (1996) indicated that organic matter reactivity changes with time, as apparent decomposition rates were substantially higher for fresh organic matter (recently deposited at the sediment surface) than those aged organic matter (buried in deeper sediments). Other experimental studies (Kristensen et al., 1995; Kristensen and Holmer, 2001) have demonstrated that fresh organic material degrade similarly under variable redox conditions while aged organic materials degrade faster under oxic than under anoxic conditions. Estuarine systems receive organic matter not only from different sources but also with different ages due to the spatial and temporal changes in the production and transport pathway of different organic matter. However, it is unclear how the ages of different organic materials affect the co-metabolism in estuarine systems. Moreover, there has been limited information on how bacteria respond to the organic matter from different sources and with different ages.

This study was designed to experimentally examine the effect of co-metabolism on degradation of organic matter in Altamaha estuarine water system. Three specific questions were addressed: (1) does the presence of labile organic material enhance degradation of refractory

organic matter? (2) does the diagenetic status (age) of allochthonous organic materials affect the degradation of labile autochthonous organic matter? and (3) how do microbial communities utilize organic carbon in estuarine systems containing organic matter from a variety of sources? We incubated different mixtures of autochthonous (fresh) and allochthonous (fresh and aged) organic materials in the estuarine water and followed variations in TOC, $\delta^{13}C_{TOC}$, material-specific fatty acids. Degradation rate constants (reactivities) of specific compounds were estimated based on a two-component kinetic model. In addition, bacterial response to varying organic matter was assessed by monitoring the variations of bacteria-specific fatty acids and the $\delta^{13}C$ ratios during incubations.

Methods

Materials. Salt marsh plant (SMP, *Spartina alterniflora*) was collected from Sapleo Island, Georgia, USA, and land grass (LG, *Festuca arundinacea*) was collected in the upland of Georgia. Marine diatom (MD, *Skeletonema costatum,* clone CCMP1332, from Provasoli-Guillard National Center for Culture of Marine Phytoplankton, USA), an abundant diatom species in the eastern US coastal water, was cultured in F/2 medium with a 12:12 light/dark cycle at a constant temperature of 16° C. The algal cells were harvested in 14 days using a centrifugation and stored temporarily at -40°C for later incubation experiments. Seawater (salinity 28‰) used in the incubation experiments was collected by pumping the surface water at the Altamaha River mouth.

After material collection, leaves of land grass and salt marsh plant were cut into small pieces (~2 × 2 mm). A portion (~10 g) of each plant material was pre-incubated separately in seawater over two months to make aged organic matter. The remaining materials were

temporarily stored at -40° C as fresh organic matter for later incubation experiments. The pre-incubation was conducted with a 12:12 light/dark cycle at 16ºC and the water was purged with air to maintain oxygenated. Detritus (aged materials) were collected by centrifugation at the end of pre-incubation.

Incubation setup. Materials were mixed (0.5 g wt of SMP; 0.83 g wt of LG; 2.5 g wt of MD, to ensure ~0.1 g dw C from each material) to form the following treatments: (1) fresh (MD + LG + SMP); (2) fresh MD + aged (LG + SMP); (3) fresh (LG +SMP); and (4) aged (LG + SMP). Each set of material mixture was equally separated into 9 portions and placed into flasks filled with 175 ml of seawater. The water in flasks was purged with air to maintain oxic conditions during the incubation (Fig. 5.1). The incubation was conducted in a temperature-controlled incubator (14±1°C) with a 12:12 (light:dark) cycle for one month. The flasks were fully shaken twice a day. At 0, 4, 8, 16, 30 days, one or two flasks (as duplicates) from each set of treatment were taken out. The materials in the water were collected by filtrating the samples through GF filters (0.5 μm, 47 mm i.d., Whatman) and then immediately stored at -40ºC for later analyses.

Total organic carbon analysis. Approximate 0.2 g material samples were treated with 10% HCl solution (sonicating in a water bath for 5 min) to remove carbonate. After air-drying in a fume hood followed by freeze-drying, the samples were ground to fine powder for bulk carbon analyses using a Carlo Erba CHN analyzer coupled with a Finnigan Delta C Stable Isotope Mass Spectrometer.

Lipid extraction and analysis. In brief, materials on filters (with filter) were extracted with 15 ml methanol, followed by 3×15 ml mixed solvents (methylene chloride/methanol, 2:1 in volume). The combined extracts (after reduction in volume by rotary evaporator) were saponified

in 0.5 M KOH/MeOH. The neutral lipids were extracted with hexane out of the basic solution (pH > 13) while the fatty acids were extracted with hexane after acidification (pH < 2). The fatty acids were then methylated to fatty acid methyl esters (FAMEs) by reacting with BF_3.

FAMEs were analyzed using a Hewlett-Packard 6890 GC with an on-column injector and a flame ionization detector. Compound separation was achieved by a 30 m × 0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5, Hewlett-Packard). Internal standards (nonadecanoic acid methyl ester) were added to samples immediately prior to GC analysis to aid in quantification. The GC temperature program was: 50-150°C at 20°C min^{-1}, followed by 150-310°C at 4°C min^{-1}, and held at the final temperature for 5 min. The compounds of interest were analyzed by a GCMS-QP5000 system (QP-5000, Shimadzu) and the structures of fatty acids were identified based on mass spectra. The GC-MS system was operated at 70 eV (ionizing energy) and the temperature program was the same as that used in the GC analysis.

Compound-specific isotope analysis. Stable carbon isotopic ratios of individual fatty acids were determined using a Varian 3400 GC-combustion system linked to a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS). The compounds were separated with a 30 m × 0.25 mm i.d. column (DB-1, J&W Scientific) and the GC temperature was programmed as: 50-150°C at 20°C min^{-1}, followed by 150-310°C at 4°C min^{-1}, and held at the final temperature for 5 min. Peaks eluting from the GC were combusted to CO_2 over CuO/Pt wires at 850°C and on-line transported to the IRMS. The isotopic compositions of CO_2 peaks were measured with the IRMS operated at 10 kV acceleration potential and by magnetic sector mass separation. The $\delta^{13}C$ ratios of the compounds were calibrated with a reference CO_2 gas ($\delta^{13}C$ = -11.23‰ relative to PDB). The standard deviation of IRMS was within ±0.67‰ based on internal standard measurements (n = 14). The $\delta^{13}C$ ratios of FAMEs were corrected for an extra carbon atom added by methylation.

Kinetics model for degradation. A two-component kinetics model, adopted from the multi-G model (Berner, 1980), was used to estimate degradation rate constants of fatty acids derived from various organic materials. It is assumed that there are two fractions, which have distinct kinetic features, for compounds bound in organic materials. The overall degradation rate is simply a sum of individual degradation rates of each fraction, as shown by the following equations:

$$dC_t/dt = -(k_1C_1 + k_2C_2)$$

$$C_t = (C_1)_0 \exp(-k_1t) + (C_2)_0 \exp(-k_2t)$$

where C_t, C_1 *and* C_2 are the concentrations of total, fraction 1 (reactive) and fraction 2 (refractory); k_1 and k_2 are the first-order degradation rate constants for fractions 1 and 2; $(C_1)_0$ and $(C_2)_0$ are the initial (at t = 0) concentrations of fraction 1 and fraction 2. In some cases, k_2 is so small (close to zero) that the fraction 2 can be assumed to be non-degradable (C_{NR}). Thus,

$$C_t = (C_1)_0 \exp(-k_1t) + C_{NR}$$

In order to directly compare overall degradation rate constants no matter how the fractions vary under different conditions, we defined a new rate constant (k_{av}), which is an average degradation rate constant based on two fractions;

$$k_{av} = k_1 \times f_1 + k_2 \times f_2$$

where f_1 and f_2 are relative proportions of fraction 1 and fraction 2 [$f_1 = (C_1)_0/(C_t)_0$ and $f_2 = (C_2)_0/C_t)_0$]. The fraction sizes $(C_1)_0$ and $(C_2)_0$ were estimated by plotting ln (C_t) vs. incubation time (t), which resulted in a breaking point to differentiate two fractions.

Results

Bulk TOC and stable carbon isotopes. Bulk TOC and stable carbon isotopes in different

treatments varied similarly during incubations (Fig. 5.2). TOC in each treatment decreased gradually and after one month, approximately 10-20% of TOC lost from various materials. Although the bulk isotopic ratios ($\delta^{13}C_{TOC}$) in different treatments were different at the start of the incubation, they all became enriched as the incubation progressed, approaching to an original $\delta^{13}C$ ratio of salt marsh plant.

Fatty acid compositions in different treatments. Fatty acid contents differed greatly in different treatments (Fig. 5.3a). Much higher fatty acid contents were observed in the treatments (1) and (2), which contained fresh MD, than in the treatments (3) and (4) without addition of fresh MD while fatty acid content in the treatment (3) with fresh LG and SMP was higher than that in the treatment (4) with aged LG and SMP. Fatty acid compositions (% of individual fatty acid relative to the total) in different treatments were also very different (Fig. 5.3b). In the treatments (1), dominant fatty acids were 14:0, 16:1, 16:0 and 18:2/18:3 (> 15% for each) while in the treatment (2), there was a little 18:2/18:3 present although 14:0, 16:1, and 16:0 were still dominant components. In the treatment (3), 16:0 and 18:2/18:3 dominated the relative compositions (>30%). In the treatment (4), there was a little 18:2/18:3 but 16:0 (>40%) and summed long chain (C_{20}-C_{28}) fatty acids (>20%) became the most important components. Based on analyses of the relative compositions in different treatments, we chose 14:0 and 16:1 as representative compounds for MD and 18:2/18:3 as representative compounds for LG and SMP. 16:0 fatty acid had a similar % relative to the total fatty acids in all treatments, so it was considered to be non-specific compound. There were several minor fatty acids (<5%) present in all treatments and we chose iso-15:0 and ante-iso-15:0 as bacteria-specific compounds to examine bacterial response to variable organic materials in different treatments.

Variations of material-derived fatty acids during incubations. Approximately 80-90% of

MD-derived fatty acids (14:0 and 16:1) degraded rapidly in the first week of incubation while remaining compounds degraded at slower rates after the first week (Fig. 5.4). It appears that the degradation proceeded relatively faster in the treatment (1) with fresh (MD + LG + SMP) than in the treatment (2) fresh MD + aged (LG + SMP). The concentrations of LG- and SMP-derived 18:2/18:3 fatty acids in the treatment (1) dropped to almost zero within one week while those in the treatment (3) declined to zero after 30 days (Fig. 5.5). Although starting concentrations were very different, the concentrations of 16:0 fatty acid (non-specific) in treatments (1), (2), and (3) decreased to a constant level over one-month incubation, which was equivalent to that in the treatment (4) (Fig. 5.6). 16:0 fatty acid in the treatment (1) degraded faster compared to those in the treatments (2) and (3). In the treatment (4), the concentration of 16:0 fatty acid was initially low and remained at a relatively constant level throughout the incubation.

Variations of bacteria-specific fatty acids during incubations. During incubations, concentrations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) varied differently in different treatments (Fig. 5.7). In the treatments (1) and (2), which contained fresh MD, the concentrations increased markedly in the first week of incubations and then gradually decreased. In contrast, the concentrations in the treatments (3) and (4), which contained only LG and SMP (fresh and aged), varied irregularly and within a small range.

Variations of $\delta^{13}C$ ratios of bacteria-specific iso-15:0 fatty acid during incubations. At the beginning of incubations, the $\delta^{13}C$ ratios of iso-15:0 fatty acid in all treatments were closely similar but varied differently among treatments during incubations (Fig. 5.8). In the treatments (1) and (2) with presence of fresh MD, the $\delta^{13}C$ ratios changed negatively (depletion) in the first week and became relatively constant afterward. By contrast, in the treatment (4) containing aged LG and SMP, the ratios changed positively (enrichment) as incubation proceeded. In the

treatment (3) containing fresh LG and SMP, the ratios varied little. During late period of incubations, the ratios among treatments were distinctly different: ~3‰ depletion for treatments (1) and (2) vs. treatment (3) and ~3‰ depletion for treatment (3) vs. treatment (4).

Discussion

Degradability of organic matter in different treatments. In this study, we incubated three organic materials in four different treatments (with and without mixing of fresh MD with fresh/aged LG and SMP). These materials had distinctly different $\delta^{13}C$ ratios (SMP: ~-12‰, LG: -30‰, and MD: -20‰, Dai et al., 2005). Therefore, the variations in stable carbon isotopic ratios of bulk organic carbon ($\delta^{13}C_{TOC}$) during incubations provided an insight into the degradability of different organic materials. In all treatments, when total organic carbon contents gradually decreased, the $\delta^{13}C_{TOC}$ became enriched, approaching to that of salt marsh plant (Fig. 5.2). This enrichment pattern suggested that isotopically lighter MD and LG were preferentially degraded over isotopically heavier SMP when organic materials coexist in an aquatic ecosystem. This conclusion is consistent with our previous studies in sediments where different organic materials were separately or together incubated (Chapter 4).

Degradation of individual fatty acids (material-specific) exhibited different patterns during incubations, also providing some clues for the relative degradabilities of different organic materials. For example, the degradation of MD-derived fatty acids (14:0 and 16:1) was characterized by a rapid decay in the first week followed by a slow decay with a small (5-10% of the initial concentration) fraction to be remained after one month (Fig. 5.4). Although non-specific (from all materials) 16:0 fatty acid also went though a similar (two stages) degradation process, the rate constants of 16:0 fatty acid was 3-4× lower than those of 14:0 and

16:1 fatty acids and a larger fraction (25-40% of the initial concentration) still remained in all treatments after one month (Table 5.1). Higher rate constants and more complete degradation of MD-derived compounds than those from all materials implied that MD might be more readily degraded than LG and SMP. Moreover, the degradability of 16:0 fatty acid in the treatment (1), where fresh MD, LG, and SMP coexisted, was apparently (>2×) higher than that in the treatment (3), where MD was absent, further confirming that MD was more labile than LG and SMP.

However, the degradability (rate constant) of 18:2/18:3 fatty acids (unique biomarkers of LG and SMP) was similar to that of MD-derived compounds (14:0 and 16:1 fatty acids) in the treatment (1) and almost completely degraded during one-month incubations in treatments (1) and (2), suggesting that there were other factors, besides organic matter source, affecting degradation of lipid compounds. Many studies (Farrington et al., 1977; Wakeham et al., 1991; Haddad et al., 1992; Bradshaw and Eglinton, 1993; Harvey and Macko, 1997) have demonstrated that polyunsaturated fatty acids are much highly susceptible to biochemical degradation than saturated fatty acids.

Effect of co-metabolism of fresh and aged organic matter on degradation. To examine the effect of co-metabolism on organic matter degradation, we compared the averaged rate constants of specific compounds between different treatments (Table 5.1). A positive co-metabolism effect was observed for 18:2/18:3 fatty acids (unique biomarkers of LG and SMP). For example, in the treatment (1), when MD coexisted with LG and SMP, 18:2/18:3 fatty acids degraded much faster (4×) than the same compounds in the treatment (3) where MD was absent. The different degradabilities of the same compound (bound in LG and SMP) between the treatments (1) and (3) implied that presence of relatively labile MD might stimulate the degradation of relatively refractory organic matter. Many studies have observed the similar positive co-metabolism effects

in soil and sediments (Smith et al., 1992; Kuzyakov et al., 2000), however the causes and mechanisms have not been well understood. A highly accepted cause is attributed to the increase of overall metabolic activity of microbes, which can enhance oxidation of originally resistant compounds (Azam et al., 1994; Lavelle and Gilot, 1994). Variations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) observed in this study indeed showed a clear evidence for stimulation of bacterial activity by addition of relatively labile MD in the treatments (1) and (2) (Fig. 5.7).

On the other hand, a negative effect of co-metabolism was also observed based on comparison of rate constants of 14:0 and 16:0 fatty acids between treatments (1) and (2) (table 5.1). The degradabilities of MD-derived 14:0 and 16:1 fatty acids were reduced by ~2× when MD was mixed with aged LG and SMP rather than with fresh LG and SMP. There were little reactive 18:2/18:3 fatty acids in the aged LG and SMP materials and the total fatty acid content in these aged materials was much lower than that in the fresh materials, showing a declined reactivity in aged materials. Similar variations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) in both treatments (1) and (2) implied that microbes might behave in the same way as long as reactive MD was present. The negative effect of co-metabolism has been observed in many studies (Sparling et al., 1982; Cheng, 1996, 1999; Wang and Bakken, 1997) and was attributed to several causes such as switch of organic matter sources for bacteria, decrease in C to N ratio, sorption or physical-chemical protection, and C-immobilization in bacterial biomass. However, we have no direct evidence to explain why the aged organic materials could reduce the degradability of fresh MD in our experimental systems.

Bacterial response to various organic matters. A variety of studies have demonstrated that bacteria would selectively utilize organic matter in natural ecosystems containing complex

substrates, depending on the relative bioavailability (bioreactivity) of different materials (Hopkinson and Vallino, 1995; Smith and Hollibaugh, 1995; Bouillon and Boschker, 2006). During growth, bacteria biosynthesize some specific compounds such as branched iso-15:0 and anteiso-15:0 fatty acids (Kaneda, 1991), which have been widely used as an indicator of organic carbon from bacterial source. In this study, the concentrations of bacteria-specific fatty acids increased immediately from the beginning of the incubations in treatments (1) and (2), where fresh MD coexisted with LG and SMP, suggesting that bacterial growth might be largely driven by relatively labile MD. However, the variations of these compounds were much less in the treatments (3) and (4), where MD was absent, implying that utilization of LG and SMP by bacteria might be hindered due to the presence of high proportion of more refractory organic compounds in these materials (Fenchel and Blackburn, 1979; Ittekkot and Lanne, 1991; Canuel and Martens, 1993). After one week of incubations when most reactive compounds degraded, the concentrations of bacteria-specific fatty acids declined, implying that bacterial growth might be outbalanced by their turnover. However, there is no simple relationship between bacteria biomass and bacteria-specific fatty acids (Harvey and Macko, 1997).

Several studies have shown that stable carbon isotope ratios of bacteria-specific fatty acids are dependent on organic carbon sources they utilize in natural ecosystems (Canuel et al., 1997; Boschker et al., 1999; Teece et al., 1999). In this study, the $\delta^{13}C$ ratios of iso-15:0 fatty acid varied differently in different treatments containing various organic materials, which help us understand how bacteria utilize different organic materials. Although the $\delta^{13}C$ ratios in all treatments were closely same (~-21‰) at the beginning of the incubations, they ended up distinctly different during late period of incubations (Fig. 5.8). In the treatments (1) and (2), where MD coexisted with fresh or aged LG and SMP, the $\delta^{13}C$ ratios were similarly at the lowest

level (~-25‰). Because the isotopic fractionation between substrate and bacteria-specific fatty acids is generally in a relatively constant range (-4 to -6‰, Boschker et al., 1999), the $\delta^{13}C$ ratios observed in the treatments (1) and (2) are likely attributed to the utilization of organic carbon from relatively labile MD, which has a bulk $\delta^{13}C_{TOC}$ ~-20‰ (Dai et al., 2005). In the treatment (4), where there were only aged LG and SMP, the $\delta^{13}C$ ratios of iso-15:0 fatty acid shifted positively during incubation and reached to a constant level (~-17‰), reflecting that a dominant carbon source for bacteria may be from SMP ($\delta^{13}C_{TOC}$ of ~-12‰, Dai et al., 2005). In the treatment (3), where fresh LG and SMP coexisted, the $\delta^{13}C$ ratios were relatively lower (~-4‰) than those in the treatment (4), indicating that both fresh LG (with a $\delta^{13}C$ of ~-29‰, Dai et al., 2005) and SMP contributed organic carbon into bacterial biomass.

Conclusions

The experimental results from this study demonstrated that either positive or negative effect of co-metabolism could function in different treatments, depending on what types of organic materials coexisted and what diagenetic statuses (ages) of the materials were. There are two aspects of implications in this study for organic matter cycling in estuarine and coastal marine systems: (1) the degradation of relatively refractory land-derived organic matter, when it enters into estuarine and coastal water, may be accelerated by a positive effect of co-metabolism with relatively labile marine phytoplankton; and (2) if the land-derived organic matter is aged (due to a long distance transport), the degradation of marine phytoplankton-derived organic matter may be retarded by a negative effect of co-metabolism. In addition, this study further confirms that bacterial metabolism is closely related to relative bioavailability (degradability) of different organic materials in a complex system such as estuarine and coastal regions.

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Table 5.1. Degradation rate constants (k, day^{-1}) of fatty acids during incubations with different material mixtures.

	k_1	k_2	f_1	f_2	k_{av}	(r^2)
14:0						
treatment 1	0.484	0.0119	0.851	0.149	**0.414**	(0.991)
treatment 2	0.202	0.034	0.937	0.063	**0.191**	(0.956)
k ratio between treatment 1 and 2					***2.161***	
16:1 (n-7)						
treatment 1	0.489	0.000	0.872	0.128	**0.426**	(0.979)
treatment 2	0.226	0.000	0.853	0.147	**0.193**	(0.891)
k ratio between treatment 1 and 2					***2.212***	
16:0						
treatment 1	0.152	0.000	0.727	0.273	**0.111**	(0.958)
treatment 2	0.100	0.000	0.770	0.230	**0.077**	(0.924)
treatment 3	0.090	0.000	0.539	0.461	**0.049**	(0.607)
treatment 4	N/C	N/C	N/C	N/C	**N/C**	
k ratio between treatment 1 and 2					***1.435***	
k ratio between treatment 1 and 3					***2.271***	
18:2/18:3						
treatment 1	0.496	0.000	1.000	0.000	**0.496**	(0.975)
treatment 3	0.124	0.000	1.000	0.000	**0.124**	(0.842)
k ratio between treatment 1 and 3					***4.000***	

treatment 1: fresh (MD + LG + SMP)

treatment 2: fresh MD + aged (LG + SMP)

treatment 3: fresh (LG + SMP)

treatment 4: aged (LG + SMP)

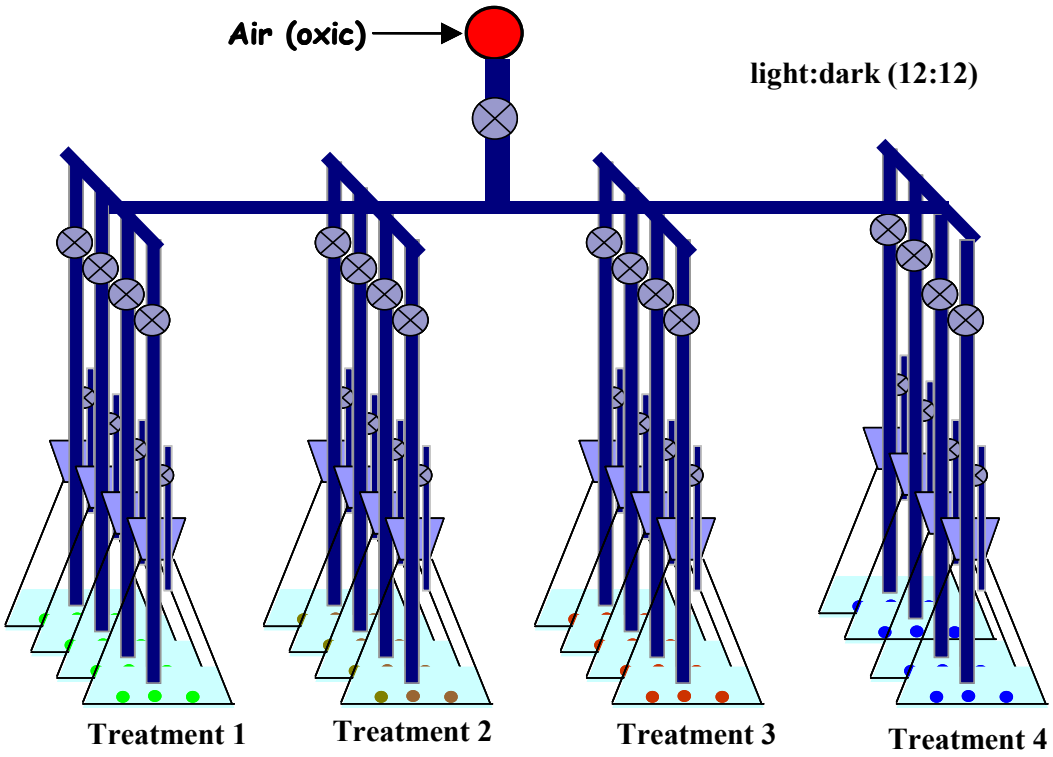

Fig. 5.1. Setup of water incubation with different material mixtures. Treatment 1: fresh (MD + LG + SMP); Treatment 2: fresh MD + aged (LG + SMP); Treatment 3: fresh (LG + SMP); Treatment 4: aged (LG + SMP).

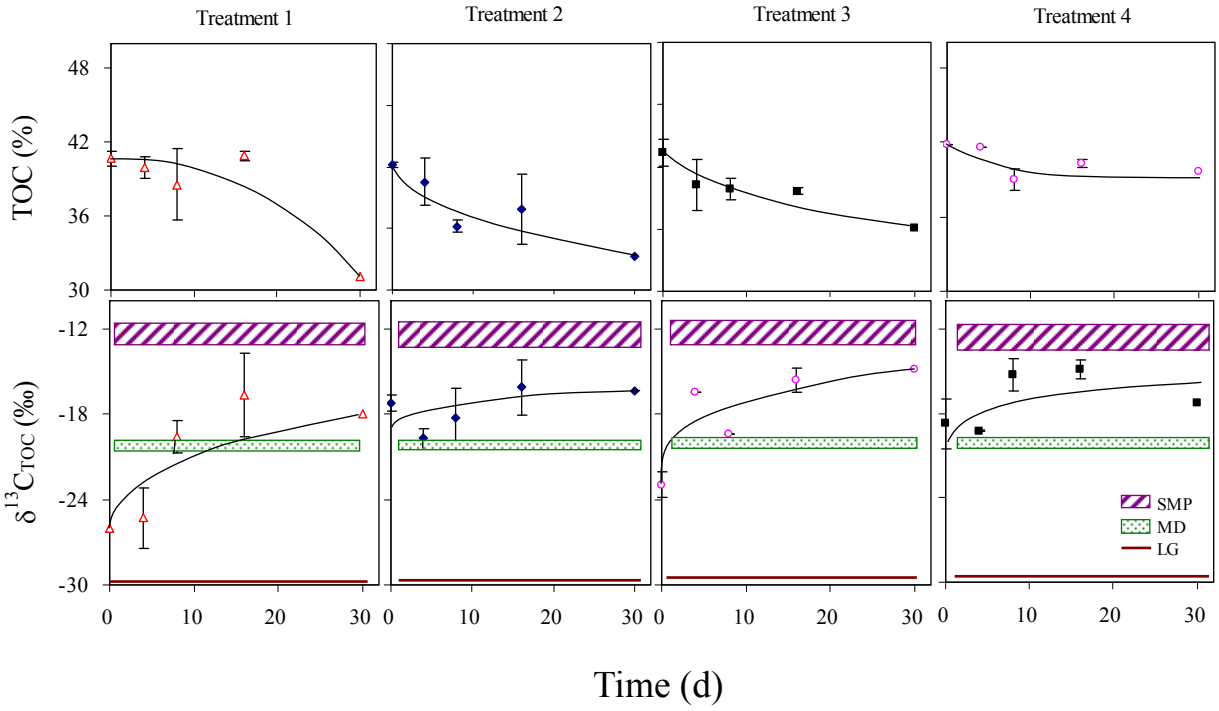

Fig. 5.2. Variations of TOC and $\delta^{13}C_{TOC}$ in different material treatments during incubations. $\delta^{13}C_{TOC}$ of three end-member organic materials (fresh and degraded salt marsh plant, marine diatom and land grass) are presented as shaded areas.

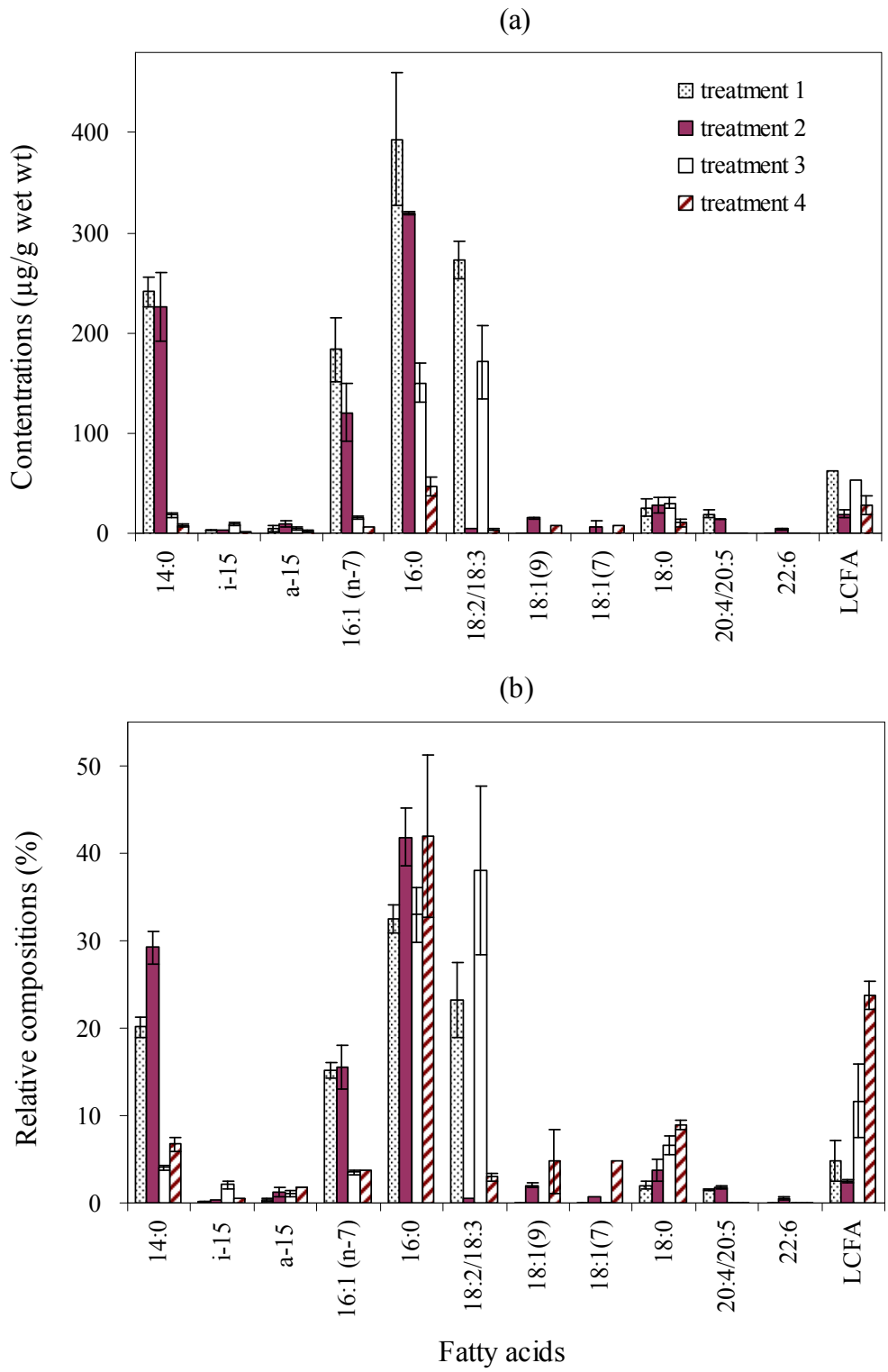

Fig. 5.3. Concentrations (a) and relative compositions (b) of fatty acids at t = 0 in different treatments during incubations.

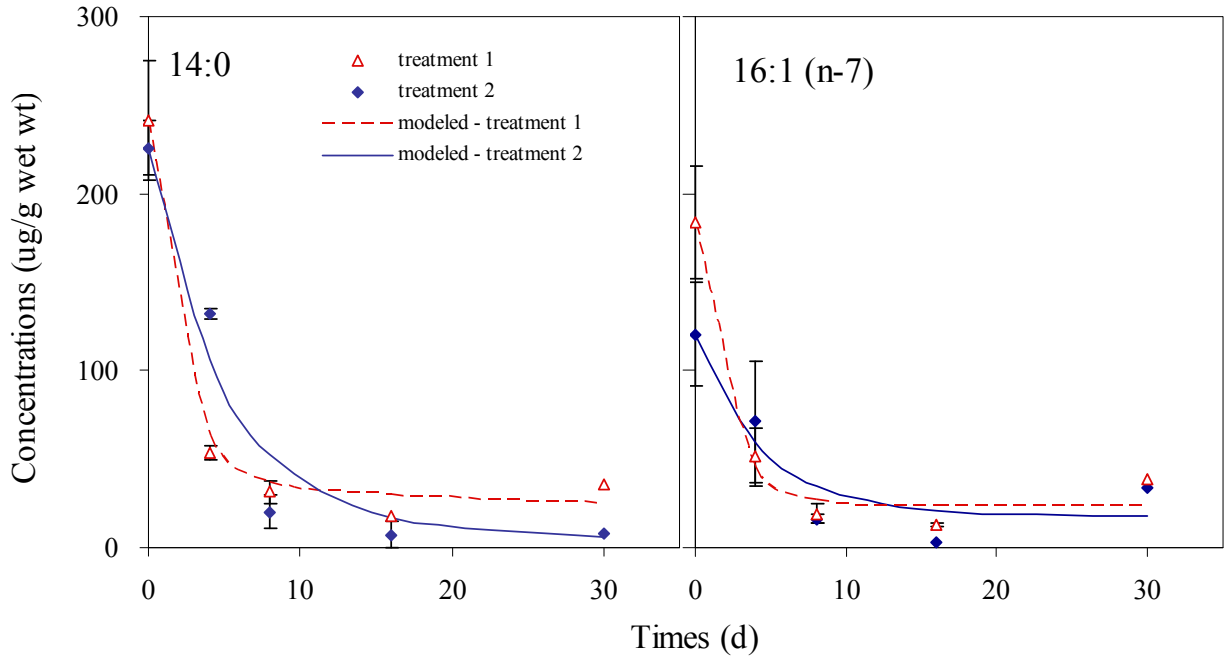

Fig. 5.4. Variations in concentrations of MD-derived fatty acids during incubations. The curves are fitting of data based on a two-component model.

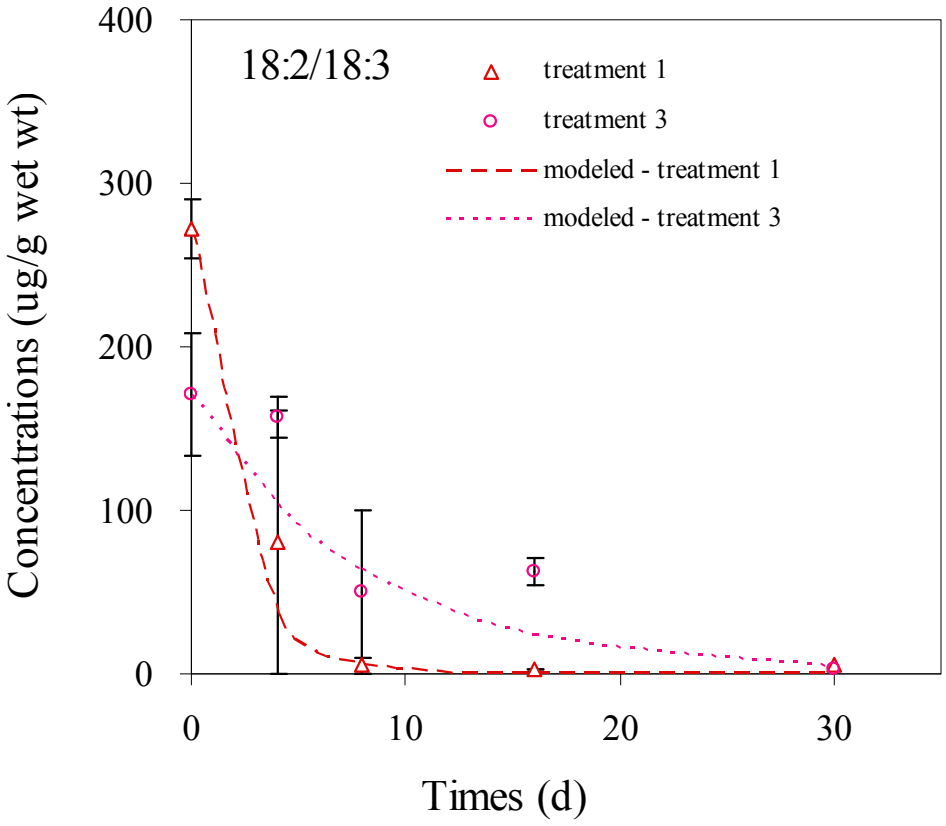

Fig. 5.5. Variations in concentrations of LG- and SMP-derived fatty acids during incubations. The curves are modeling results.

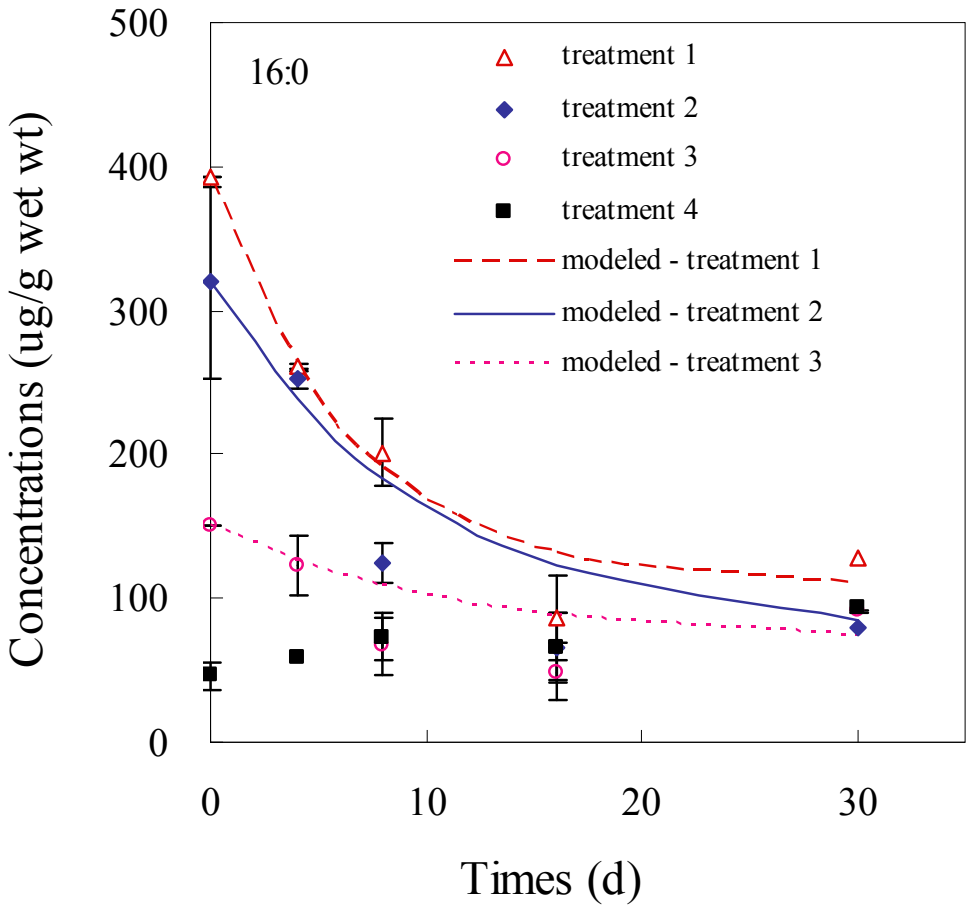

Fig. 5.6. Variations in concentrations of non-specific (from all sources) 16:0 fatty acid during incubations. The curves are modeling results.

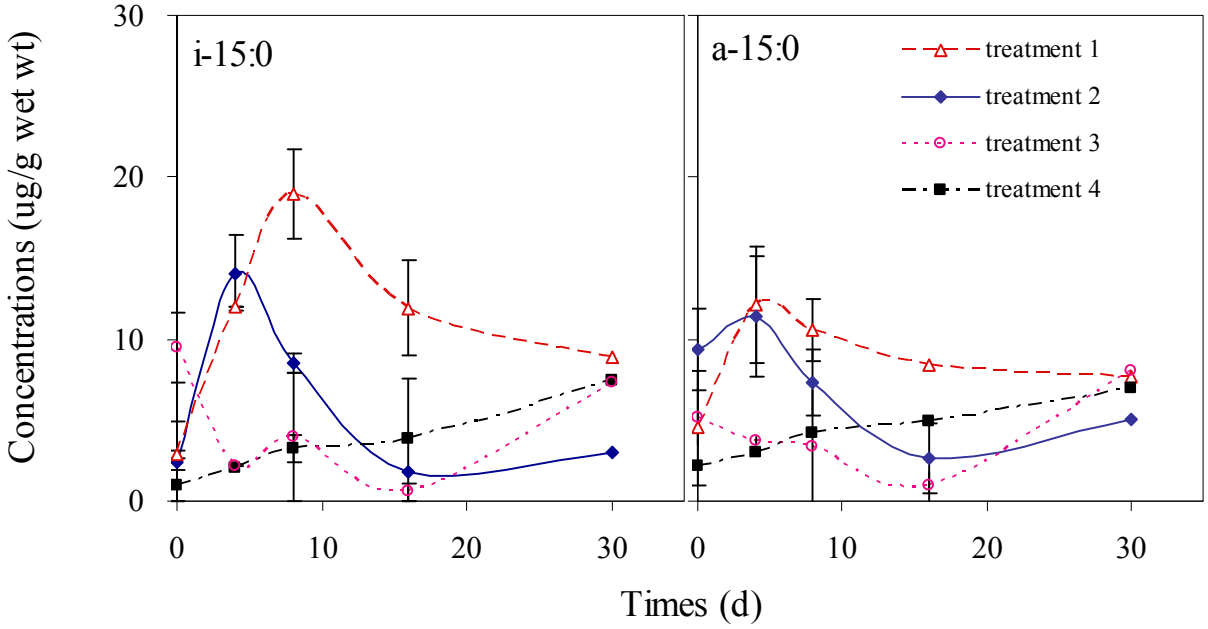

Fig. 5.7. Variations in concentrations of bacteria-specific fatty acids (iso-15:0 and anteiso-15:0) during incubations.

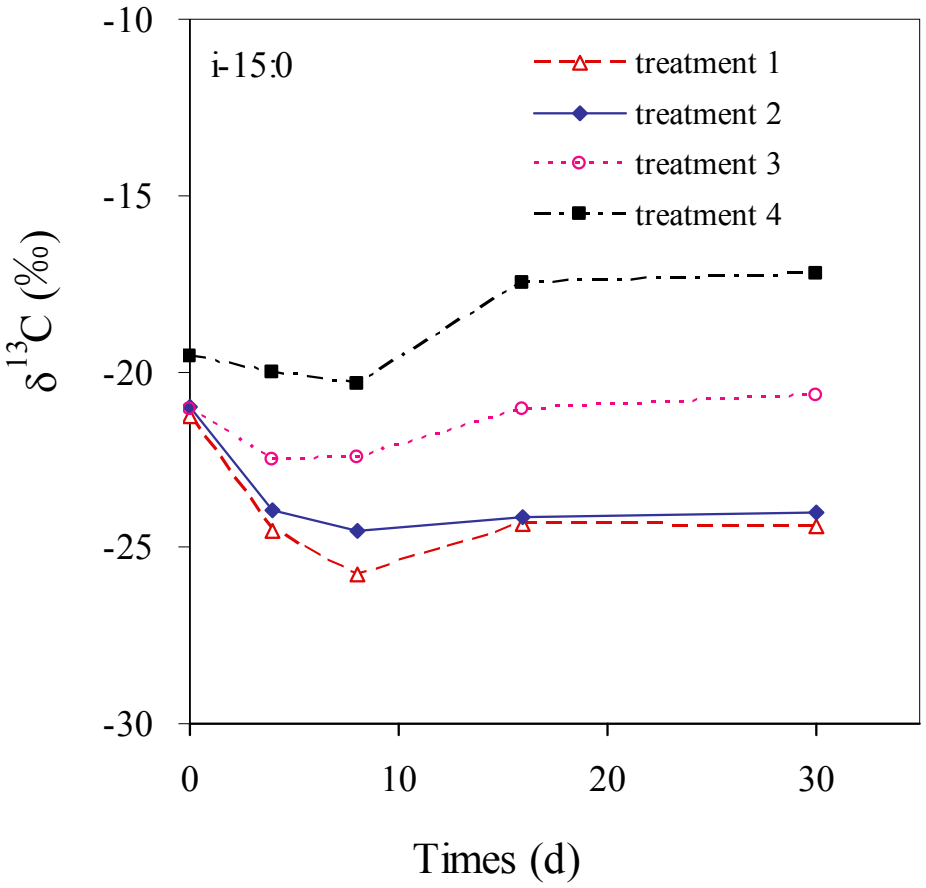

Fig. 5.8. Variations in $\delta^{13}C$ of bacteria-specific fatty acid (iso-15:0) during incubations.

CHAPTER 6

GENERAL SUMMARY

Estuaries possess overriding importance of organic matter production and degradation in biogeochemical carbon cycling. However, our understanding regarding sources and behavior of organic matter in this system remains incomplete. A major cause for this is that complicated biochemical processes in estuarine systems can not be understood by using a single technique or by field distribution study alone. Combination of multiple techniques and combination of field measurements and laboratory simulation experiments will no doubt be a real solution. In this study, I applied chemical and isotopic analytical approaches to determine bulk parameters and molecular signatures of organic matter in intensive laboratory simulations as well as field observations. Three-end-member model and Principal-Component Analysis were applied in an attempt to understand fluctuations in the delivery of organic matter to the estuarine systems during different discharge periods. A two-component degradation model was also applied to determine the rates of organic matter degradation by microbially-mediated processes. Although these issues are discussed in separated chapters, they are intimately related.

Changes in chemical and isotopic signatures during early diagenesis. Major end-member materials in the Altamaha estuary are characterized by distinct chemical and isotopic information. However, these compositional signals are subject to changes when exposed to degradation in estuarine water and sediment. The main processes involved include organic matter

remineralization, selective preservation of isotopically distinct fractions of organic matter and bacterial biosynthesis. This study indicates that although the bulk isotopes changed in a small range (<2‰) in different materials, the isotope compositions of major lipid biomarkers (fatty acids and neutral lipids) could be significantly different before and after the degradation.

The potential changes in chemical and isotopic signatures during degradation processes suggest that application of end-member model to trace the relative contribution of organic matter from different sources should be used with consideration of diagenetic status of different source substrates in the system. When we presented our observation in a more quantitative manner, freshness of end-member materials could be assessed from representative molecular ratios, such as 16:1/16:0 in marine diatom and 18:2+3/16:0 in plant materials, before and after the decomposition. Isotopic abundance coupled with certain freshness status of materials could thereafter be estimated by doing a simple mass-balance calculation.

In spite of the degradation alterations, trends in isotopic compositions of major compounds in different end-member materials, however, kept consistency with isotopic abundance of total organic matter as expected for C3 and C4 materials, implying promise of using isotopic ratios of material-specific compounds in revealing source identity of organic matter.

Effect of riverine discharge on distributions of organic matter from different sources in the Altamaha River estuary. The Altamaha River is an important drainage basin on the Georgia coast, with a remarkable seasonal variation in discharge. Analysis of bulk organic carbon and lipid biomarkers associated with surface sediment (0-2 cm) along the Altamaha transect reveals trends in the relative importance of various carbon sources as a function of riverine discharge: during the high discharge period (March), inputs from C3 terrestrial plants and marine phytoplankton were dominant in the sediments but land-derived organic matter deposited mostly

at the upstream sites of the river, while during the low discharge season (October), C4 salt marsh plants contributed a large fraction of organic matter at some specific site.

End-member modeling and Principal-Component Analysis (PCA) consistently confirm the variation patterns of allochthonous and autochthonous inputs during different discharge periods. Moreover, PCA results demonstrate that organic matter in the high discharge period was relatively fresh while that in the low discharge period was highly degraded. The difference in diagenetic status of organic matter during different seasons can be attributed to the changing metabolic activities at different temperatures. On the other hand, the algal materials were readily decayed compared to the terrestrial materials, resulting in low compositions of algal lipids deposited in sediments during low discharge period despite a large fraction of organic matter from aquatic production.

Factors controlling bioreactivity of organic matter in estuarine ecosystems. Organic materials have different bioreactivities that result in differential utilization of components by the bacterial community. Previous researches demonstrate that aquatic and marine algae are readily degraded while terrestrial (or vascular) plants are more resistant to microbial attack due to a greater amount of recalcitrant macromolecules such as lignin, tannin, suberin and cutin in higher plants bound in these materials. This study further elucidates the order of biochemical reactivity of typical end member materials in the Altamaha estuarine systems: marine phytoplankton > land grass > salt marsh plant. The high degradation rate constants occurred in marine diatom-derived organic compounds rather than salt marsh-derived compounds, suggesting that the carbon source is one important factor controlling the reactivity of the organic matter in estuaries.

Interestingly, polyunsaturated fatty acids 20:4/20:5 and monounsaturated fatty acid 16:1(n-7) were more completely and rapidly degraded than saturated fatty acid 14:0 even though

they all come from marine diatom, indicating that material sources cannot be solely responsible for the bioreactivity of organic matter. Instead, structural feature (i.e., unsaturation) plays a critical role in degradation of organic matter. The reason could be traced to the different susceptibilities of molecules to degrading enzymes within material matrixes. The higher proportion of highly-resistant compounds in terrestrial materials than in marine phytoplankton may prevent microbial enzyme access and thus greatly slow down the removal of carbon from the metabolizable pool.

It was expected that organic matter degradation rates under oxic conditions would be higher than those under anoxic conditions. Yet, in our incubation studies of different fresh materials in aged sediments, only a small difference in degradation rates was noted between oxic and anoxic conditions. It is likely that the bacterial communities that survived after the 6-month pre-incubation were facultative so that they remineralized the organic matter at a comparable rate in both systems.

Many studies have demonstrated that the degradation of labile components can accelerate decay of more refractory components, which is likely caused by co-metabolism. The co-metabolism, however, leads to different results on organic matter degradation in this study. It seems that the effect of co-metabolism is dependent on the type of organic materials coexisting in the system and the diagenetic status of the materials. Because degradation of organic matter is also primarily microbially-mediated, the function of bacteria and their interactions with different organic materials might be responsible for the results in different material treatments. However, there are few studies which clearly explain the causes and mechanism of co-metabolism.

Bacterial utilization of organic matter in estuarine systems. Bacterial-mediated diagenetic transformation of organic matter is one important process that alters chemical and isotopic

signatures of source materials. In oxygenated water incubations with single end-member materials, the bacteria-specific fatty acids iso-15:0, anteiso-15:0 and 18:1(n-7) showed $\delta^{13}C$ compositions close to those of substrate materials and remained distinguishably different in different material, indicating that $\delta^{13}C$ of bacteria-specific fatty acids can be used to trace the carbon source of microbial communities. In sediment incubation experiments, the concentrations of bacteria-specific fatty acids increased rapidly in all treatments with addition of single substrate, suggesting that bacteria rapidly responded to addition of any fresh organic materials after being pre-incubated for six months. Interestingly, in treatments where the three materials were mixed together, the $\delta^{13}C$ ratios of bacteria-specific fatty acids were closely similar to those of marine diatom treatment, implying that bacteria indeed preferentially utilize the most reactive organic carbon when a variety of organic materials coexist in the system.

The selective incorporation of organic matter from algal source by bacterial communities was confirmed by the field observation where bacterial fatty acids followed the same patterns as Chl-a and algal lipids in both high and low discharge seasons but showed little relationship with terrestrial lipids. However, when salt marsh plants became an important input into the sediments during the low discharge period, bacteria efficiently used this organic matter, as indicated by the coincident occurrence of unusually enriched $\delta^{13}C$ ratios of both TOC and bacteria-specific fatty acids. The inconsistency could be at least partially attributed the different microbial metabolism at different temperature. During the low discharge period (higher temperature), the bacterial activity was stimulated, resulting in more degraded algae-derived organic matter deposited in the surface sediments even though the phytoplankton production was high at this time. Consequently, bacteria used the freshly salt marsh plants rather than the degraded (less labile) algal components. Further support for the importance of diagenetic status in controlling bioavailability of organic

matter is seen by the high degradation rate constants in treatments with all fresh materials compared to those with addition of aged materials.

Significance of this study and outlook. Overall, the remineralization of organic matter involves a complex interaction between the type of organic matter, environmental conditions and microbial consortia. The results from this study help us draw a general picture for variations of organic matter inputs in the Altamaha estuary. In addition, the study produced substantial information regarding the environmental controls on biochemical reactivity of different organic material and provided insights into interaction between organic matter and microbial communities. We hope that our results may trigger further and deeper research of organic matter cycling in estuarine systems. One challenge in better quantifying the contribution of organic matter from different sources will be to identify other potential sources. For example, influences of freshwater phytoplankton, benthic phytoplankton and terrestrial species other than land grass and salt marsh plants may need to be considered in the end-member modeling. To better understand organic matter degradation and the microbial community responsible for this process, an important future direction will be to simultaneously observe the dynamics of organic matter, bacterial abundance and enzyme activity. Moreover, use of isotopically labeled tracers may give the possibility to state clearly the causes and mechanisms of co-metabolism effect.