Lipid-degrading enzyme activities associated with distribution and degradation of fatty acids in the mixing zone of Altamaha estuarine sediments

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Abstract
To examine the relationship between lipid-degrading enzyme activities and lipid composition in estuarine sediments, lipase activities and fatty acids in surface sediments at different stations along the Altamaha River were determined during a high discharge period. Freshwater sandy sediments from upstream stations were characterized by low organic carbon and low lipid contents. Sediments from the strong mixing zone, where salinities were highly variable, were characterized by high organic carbon and high lipid contents with a predominance of long chain (>C20) saturated fatty acids, derived from terrestrial plants. At the river mouth, total fatty acids in the sediments were at intermediate concentrations, with a predominance of terrestrial plant fatty acids. Lipase activity in the Altamaha River sediments was associated with certain fatty acid subgroups, i.e. monounsaturated, short-chain saturated, and branched-chain fatty acids. This indicates that these lipases probably were produced by bacteria. There was no significant correlation between lipase activity and terrestrial plant fatty acids (long-chain saturates) or algal fatty acids (polyunsaturates). A 3 month incubation of Altamaha River sediment, collected from the strong mixing zone, was carried out to determine changes in lipase activities and degradation rates of the various fatty acids. Solvent-extractable fatty acids were more readily degraded than bound fatty acids (fatty acids released from the extracted-sediment residue by saponification). Chain length and degree of unsaturation, which were linked to the lipid source, affected fatty acid degradation rates. The presence of water overlying sediment resulted in increased sediment lipase activity during incubation, presumably due to stimulation of microbial processes at the sediment–water interface. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lipase activity; Extractable and bound fatty acids; Incubation experiment; Correlation between lipase and fatty acids

1. Introduction
Organic matter from a variety of sources is trapped in estuaries with rapid microbial degradation of labile components taking place at the sediment surface, which results in remineralization and regeneration of inorganic carbon and nutrients (Rowe et al., 1975; Klump and Martens, 1981; Azam, 1998). Particulate organic matter reaching the sediment–water interface includes macromolecular aggregates composed of carbohydrates, proteins and lipids, which are degraded by extracellular enzymes and subsequently incorporated into microbial biomass (Meyer-Reil, 1991; Arnosti et al., 1994; Arnosti, 1998). A rate limiting step in the degradation of organic matter is the enzymatic hydrolysis of the macro-molecules (Billen, 1982; Burns, 1983; Meyer-Reil, 1987). Protein- and carbohydrate-degrading enzymes in sediments have been well-investigated (Ladd and Butler, 1972; King, 1986; Mayer, 1989; Billen, 1991; Mayer and Rice, 1992; Arnosti, 1995,1998; Mayer et al., 1995). Fewer studies have dealt with the relationship between lipid-degrading enzyme activities and lipid degradation rates in sediments.

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Extracellular enzymes, which act outside the cell or are located on the outer cell membrane, are produced by bacteria (Corpe and Winters, 1972) and are released during the lysis of dead and decaying cells. Some enzyme activity may be retained due to the formation of humic-enzyme complexes bound to clay particles (Burns, 1980). Enzyme activity is affected by physical, biological, and chemical processes in the environment. For example, sediment resuspension by storms resulted in increases in enzymatic degradation rates of sediment organic matter (Chrost and Riemann, 1994). Degradation enzyme activities were high in the water and sediments during phytoplankton blooms (Reichardt, 1986; Meyer-Reil, 1987; Gajewski and Chrost, 1995). Biogenic structures in sediments, e.g. tubes and burrow walls of macrofauna, are associated with high microbial activity due to physical disturbance of the sediment (Eckman, 1985; Findlay et al., 1985) or infaunal metabolism (Yingst and Rhoads, 1980; Alongi and Hanson, 1985). Redox changes in estuarine sediments are associated with changes in the activities of various hydrolytic enzymes (Reichardt, 1986). Further study is needed on the relationship between degrading enzyme activities and organic carbon cycling in sediments.

Lipids are an important class of natural organic matter and have been widely used as biomarkers in geochemical studies on the source, fate, alteration and historical changes of organic matter (Gagosian et al., 1980; Mackenzie et al., 1982; de Leeuw and Bass, 1986; Lee and Wakeham, 1988). Lipids deposited in different environments degrade at different rates (Haddad et al., 1992; Sun and Wakeham, 1994; Canuel and Martens, 1996; Jeng et al., 1997). Lipid degradation rates in sediments depend on the reactivities of these compounds in the natural sediment matrix and the effectiveness of lipid-degrading enzymes. Fatty acids in organic matter are generally esterified in wax esters, triacylglycerols, phospholipids and sterol esters. It is not clear how lipid-degrading enzymes, e.g. lipase, deal with these different lipid complexes as they enter sediments. Lipase hydrolyzes emulsified long-chain tri-, di- and mono-glycerides, releasing free fatty acids. One lipase assay is based on the hydrolysis of the colorless substrate p-nitrophenylpalmitate (PNPPal), to yield the yellow end product, p-nitrophenol (Gajewski et al., 1993). Although this spectrophotometric method has been widely applied in measuring lipase activities in water samples (Boon, 1989; Gajewski et al., 1993; Gajewski and Chrost, 1995), there are few reports of its use in sediment samples.

The present study focused on the relationship between lipase activities and sedimentary fatty acid cycling in an estuarine mixing zone in the Altamaha River, GA, USA. Two questions are asked: (1) what is the relationship between lipase activities and concentrations of different fatty acid subgroups, and (2) what is the relationship between lipase activities and fatty acid degradation rates. Two approaches were taken to determine if changes in lipase activities were correlated with changes in fatty acid distribution and degradation rates in sediments: (a) determination of fatty acid composition and lipase activities in surface sediments from different stations in the Altamaha River; (b) determination of degradation rates of various fatty acids and lipase activities in laboratory incubated sediments collected from a strong mixing zone in the Altamaha River.

2. Experimental

2.1. Study site and sediment sampling

The Altamaha River is the largest river in Georgia and the discharge ranges from >1500 m³/s (spring) to <70 m³/s (fall). The bedload sediments are derived from igneous and metamorphic rocks of the Piedmont and from sedimentary rocks of the coastal plain. The upper coastal plain is an intensely cultivated region in Georgia but there is relatively little industrial, commercial or residential development. The hydraulic gradient of the Altamaha River drops substantially in the last 50 km to less than 5 cm/km. The daily tidal range is approximately 2 m and brackish water can reach 36 km upstream during late summer–early fall. Combined marine and riverine processes have resulted in the establishment of a diverse plant community in the study area (Gallagher and Reimold, 1973; Schubauer and Hopkinson, 1984). Note that there is little development along the Altamaha and thus minimal sewage discharge into the river.

Sediment samples were collected by using a spade corer at seven sites along the Altamaha River mixing zone (between the mouth and upstream 20 km) in early April 1998 (Fig. 1). Water depths at these sampling sites vary from 3 to 10 m and salinities also change with distance towards the mouth and during tidal cycle; the sediment particles at these sampling sites are dominated by sand but organic content has a great variation among these sites (Table 1). Two types of sediment samples were collected: surface sediment samples (0–2 cm) in entire mixing zone, and one core (0–10 cm) at station 5N. After collection, surface samples were frozen for later analyses of lipase activity and lipids. Core samples (0–10 cm interval) were homogenized by hand on the boat and split into glass bottles (230 ml). Each bottle contained approximately 20 g of homogenized sediment which was covered with ~200 ml water collected from the same site. Sediment samples were analyzed for lipids and lipase activity.

2.2. Incubation setup

Incubation experiments with sediment from station 5N were carried out in the dark with temperature
maintained at 21.5±1°C, which was the in-situ temperature at the sampling site. At various time intervals (0, 7, 21, 36, 49, 70 and 92 days), bottles were removed from the incubator. Water in each bottle was removed and wet sediment was used for lipase activity measurements and lipid analyses.

2.3. Fatty acid extraction and analysis

Procedures for extraction and analysis of sedimentary lipids were previously described (Sun and Wakeham, 1994; Sun et al., 1997). About 0.5 g of the wet sediment was dried at 60°C for 24 h to measure water content and approximately 5 g of wet sediment was extracted with 10 ml methanol, followed by 3×10 ml methylene chloride–methanol (2:1 v/v). During the extraction, samples were sonicated for 10 min. Combined extracts were partitioned into a methylene chloride phase formed by the addition of 5% NaCl solution and the volume reduced to 1 ml with a rotary evaporator. This extract will be referred to as “solvent-extractable lipid pool”. The extracted sediment residue was subsequently saponified with 0.5 M KOH for 2 h and filtered. The resulting filtrate will be referred as the “bound lipid pool”. Lipids in the solvent-extractable pool were further saponified using 0.5 M KOH. Neutral lipids in both solvent-extractable and bound pools were extracted from the basic solution (pH > 13), and acidic lipids were extracted after addition of HCl (pH < 2).
Table 1
Salinity (surface and bottom waters), organic carbon content, and particle type (relative percentage) at seven stations in Altamaha River

<table>
<thead>
<tr>
<th>Station</th>
<th>Depth (m)</th>
<th>Salinity (%) in low tide surface bottom</th>
<th>Salinity (%) in high tideb</th>
<th>OC (%)</th>
<th>Sand %</th>
<th>Clay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1N</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.02</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>2N</td>
<td>10.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.02</td>
<td>98.8</td>
<td>1.2</td>
</tr>
<tr>
<td>3N</td>
<td>6.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.02</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>4N</td>
<td>6.9</td>
<td>0.0</td>
<td>0.0</td>
<td>1.36</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>5N</td>
<td>3.5</td>
<td>3.9</td>
<td>4.4</td>
<td>0.55</td>
<td>n/a</td>
<td>95.6</td>
</tr>
<tr>
<td>6N</td>
<td>3.9</td>
<td>4.5</td>
<td>7.5</td>
<td>0.03</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>7N</td>
<td>2.2</td>
<td>4.6</td>
<td>14.1</td>
<td>0.02</td>
<td>100</td>
<td>0.0</td>
</tr>
</tbody>
</table>

a Source: M. Molina, unpublished data.

b Data from cruise on 28 March 1997.

Fatty acids in the acidic lipid extracts were converted to fatty acid methyl esters (FAMEs) by reaction with BF3-MeOH at 100°C for 2 h. FAMEs were analyzed by capillary gas chromatography using a Hewlett-Packard 6890 GC with an on-column injector and flame ionization detector. Separations were achieved with a 30 m × 0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5) operated with a temperature program of 80–150°C at 10°C/min followed by 150–310°C at 4°C/min and a 5 min hold at 310°C; hydrogen was the carrier gas. A quantification standard (non-adecanoic acid methyl ester; not present above detection limits in any sample) was added to samples prior to GC analysis. Some samples were analyzed by gas chromatograph–mass spectrometry (GC–MS) to help in the identification of fatty acids. GC–MS was performed on a Shimadzu QP-5000 GC–MS system using a 30 m × 0.25 mm i.d. column coated with 5% phenyl methyl silicone (XTI-5, Restek) and helium was used as carrier gas. Operating conditions were: mass range 50–610 with a 0.4 s scan interval; 70 eV ionizing energy; GC temperature program 50–150°C at 20°C/min followed by 150–310°C at 4°C/min and a 5 min hold at 310°C. Relative standard deviations of fatty acid analysis was less than ±5% based on duplicate measurements.

2.4. Lipase activity assays

To avoid high background color levels resulting from DOC or humics, enzymes were extracted from sediments prior to determining enzyme activities. Lipase was extracted from ~5 g of wet sediment with 3×1.7 ml solution of 2% Triton-X100 containing 2 g/l of polyvinylpyrrolidone (PVP) in 0.2 M Tris–HCl buffer (pH=8.0) (Reichardt, 1986). Each extraction was carried out in ice water for at least 10 min, with continuous shaking by hand. Extraction efficiency was checked by measuring absorbance in each extract: absorbance derived from the third extract was less than 15% from total extract. Extracts were combined and split into several portions, then mixed with a series of different concentrations of substrate solutions. The stock substrate solution of PNPPal was 100 mM in 95% ethanol. For enzyme assays, PNPPal substrate solutions were added to the sub-extracts, yielding final substrate concentrations of 10, 25, 50, 100, 250 and 500 μM in the assays.

Immediately after substrate addition, the absorbance of samples was measured for time zero results. Samples were then incubated at 21.5°C (same as in-situ sampling temperature) for 10 h in the dark. The absorbance of the yellow product was determined at 410 nm using a Shimadzu UV-2501 PC spectrophotometer. PNPPal often creates an emulsion in the aqueous solution, so that samples for lipase assays have to be filtered through GF/F filters before reading the absorbance (Gajewski and Chröst, 1995). However, in our studies, emulsions were not formed since Triton X-100, PVP and Tris–HCl buffer were added to sediment extracts to avoid this problem. The addition of different substrate concentrations to sediment extracts allows determination of enzyme–substrate saturation and the calculation of kinetic parameters of lipase in the sediment extracts. The enzymatic reaction rate was calculated according to the following equation:

\[ v \text{ (nmol cm}^{-3}\text{ h}^{-1}) = \frac{(\Delta A/\Delta t) \cdot [(V_{cell} \cdot V_i)/(\varepsilon \cdot V_{sed})] \times 10^6}{ } \]  

(1)

where \( \Delta A = \) absorbance change; \( \Delta t = \) incubation time (h); \( V_{cell} = \) spectrophotometer cell volume (4 ml); \( \varepsilon = \) molar extinction coefficient (15772 l mol\(^{-1}\) cm\(^{-1}\); Eisenthal and Danson, 1993); \( V_i = \) total volume of sediment extract (μl); \( V_{sed} = \) volume of sediment extract added to the cell (500 μl in this case); and \( V_{cell} = \) total volume of sediment (cm\(^3\)). Due to the presence of naturally-occurring substrates and competition with added substrates for enzyme sites, the measured rates in this study are “potential” rate.
Enzymatic reactions generally follow Michaelis–Menten kinetics and the plot of the rate ($v$) vs. substrate concentrations $[S]$ results in a rectangular hyperbola:

$$v = \frac{v_{\text{max}}[S]}{(K_m + [S])}$$

where $v_{\text{max}}$ is the maximum rate of the enzyme reaction which is theoretically attained when the enzyme has been saturated by an infinite concentration of substrate $[S]$, and $K_m$, the Michaelis constant, which is numerically equal to the concentration of substrate for the half-maximal rate. The values of $v_{\text{max}}$ and $K_m$ can be estimated by plotting $(1/v)$ vs. $(1/[S])$. However, the lipase reaction rates and substrate concentrations determined in this study often did not follow Michaelis–Menten kinetics. Lipase activity responded slowly to the change of substrate concentration at low concentration range. The reason for the initial lag in the enzyme reaction was not clear. An alternative solution is to use the Hill equation (Eisenthal and Danson, 1993):

$$v = \frac{v_{\text{max}}[S]^h}{(K_m + [S]^h)}$$

Comparing with the Michaelis–Menten equation, a new parameter $h$ (the Hill constant) is introduced. When $h$ equals 1, the Hill equation will be simplified to Michaelis–Menten equation. The Hill equation can be transformed to a linear relationship as:

$$\log \frac{v}{v_{\text{max}} - v} = h \cdot \log [S] - \log K_m$$

In this study, the enzyme reaction rate vs. substrate concentration could be fitted to the Hill equation using software program (Winnonlin 1.1, Pharsight). The kinetic parameters ($v_{\text{max}}$ and $K_m$) were calculated from a nonlinear regression analysis of $(v)$ vs. substrate concentration $([S])$. The Hill constant (slope of the line) was found to be always greater than 1. That represents a kinetic behavior called positive co-operativity. Positive co-operativity is a phenomenon reflecting the equilibrium binding of substrates where the binding of one molecule of a substrate to an enzyme can facilitate the binding of subsequent molecules of the same substrate. Relative standard deviations of lipase activity assays were generally ±5% ($n=4$).

3. Results

3.1. Effect of sample storage on sediment lipase activity

To test the effect of freezing of sediment samples on lipase activity, the following samples were used for lipase assays: (1) fresh sample (<1 day after collection); (2) frozen sample (>15 days); (3) samples stored at room temperature (15 days). These sediment samples were taken from same site in the Altamaha River. Frozen (−40°C) and fresh samples showed the same absorbance increase pattern when their extracts were mixed with substrate (Fig. 2). When the fresh sample (without overlying water) was kept at room temperature for 15 days, the sediment showed very little lipase activity. Thus, sediment freezing did not affect lipase activity and sediments collected in the field were frozen for later lipase assays without significant loss of activity.

3.2. Distribution of fatty acids in surface sediments

Total identified fatty acid concentrations (sum of 19 major fatty acids) in surface sediments (0–2 cm) varied at the different Altamaha River stations (Fig. 3). Fig. 1 and Table 1 show the location and describe some physical and chemical properties of the stations sampled in the Altamaha River. Stations 1N–3N contained freshwater sandy sediments characterized by low organic carbon. Stations 6N and 7N located in the mouth of the river with higher salinities had sandy sediments with low organic carbon content. Stations 4N and 5N contained sediments with the highest organic carbon and a significant amount of clay at station 5N. Salinities between 4N and 5N range from 0 to 20% during a tidal cycle (Cai and Wang, 1998), thus this region of the river is a strong mixing zone. The highest fatty acid concentration (~36 μg/g, including both solvent-extractable and bound forms) was at station 4N, where the ratio of bound to solvent-extractable fatty acids was greater than one. Much lower fatty acid concentrations (3–8 μg/g) were found at stations 1N, 2N, 3N and 5N, where...
there were roughly equal amounts of solvent-extractable and bound lipids. At stations 6N and 7N (near the river mouth), fatty acid concentrations were 2–3 times higher than those at stations 1N, 2N, 3N and 5N, and there were relatively more solvent-extractable than bound lipids. In this study, the definitions of fatty acid pools were based on the method of extraction (see Experimental). The fatty acids in the solvent-extractable pool are primarily present as various esters and complexes rather than free fatty acids. The fatty acids in the bound pool are more likely linked with sediment matrixes rather than free fatty acids. The fatty acids in the bound pool are more likely linked with sediment matrixes which resist extraction by organic solvents.

Fatty acid subgroups had different distribution patterns at the different sampling sites. Long-chain (>C₂₀) saturated fatty acids predominated at station 4N, where there was a higher portion of bound vs. solvent-extractable lipids (Fig. 4a). Sediments at the other six sampling sites had only trace amounts of long-chain saturated fatty acids. Polysaturated (18:2, 20:5, and 22:6) fatty acid concentrations were highest at station 6N and 7N, which were characterized by higher solvent-extractable than bound lipids (Fig. 4b). At the upstream stations (1N and 2N), there were only trace amounts of polysaturated fatty acids. From the upstream sites to the river mouth, polysaturated fatty acid concentrations gradually increased. Because of intensive dynamics in the estuarine mixing zone, concentrations of fatty acids in the surface sediments do not represent net deposition. However, there were important differences in the distributions of long-chain saturated and polysaturated fatty acid concentrations at the different sites.

Three other subgroups of fatty acids, including C₁₆–C₁₈ monounsaturated (MUFA), short-chain (C₁₄–C₁₈) fatty acids (SCFA), and branched-chain (iso- and anteiso-15:0) fatty acids (BCFA), had similar concentration patterns at the seven sampling sites (Fig. 4c–e). Higher surface concentrations of these fatty acids were found at station 4N, 6N and 7N, dominated by the solvent-extractable lipids. Lower concentrations of these fatty acids were found at stations 1N, 2N, 3N, and 5N, with a ratio of one for bound to solvent-extractable lipids.

3.3. Lipase activity in surface sediments along the Altamaha River

Very different lipase activities were found in surface sediments from the different stations (Table 2). Highest maximum rate of enzyme reaction, \( v_{\text{max}} \), was found at station 4N (67.8 nmol cm⁻³ h⁻¹) while the lowest \( v_{\text{max}} \) was at station 3N (18.0 nmol cm⁻³ h⁻¹). \( v_{\text{max}} \) ranged from 25 to 45 nmol cm⁻³ h⁻¹ in sediments from the other five stations. The highest Michaelis constant, \( K_m \), was at station 4N (331 μM) while the lowest was at station 1N (117 μM). Lipase \( v_{\text{max}} \) correlated with mono-unsaturated, short-chain and branched-chain fatty acid concentrations (Fig. 5; regression coefficient \( r^2 = 0.6–0.8 \)) at the different sites. No correlation was found between lipase \( v_{\text{max}} \) and polynsaturated or long-chain saturated fatty acid concentrations.

3.4. Fatty acid degradation rates in laboratory sediment incubations

Fatty acid degradation rates were determined during laboratory incubation of sediments from station 5N. Decreases in fatty acid concentration were fitted using a least square regression of the simple first order kinetic equation:

\[
C_t = C_0 \exp(-kt)
\]

(5)

where \( C_t \) = the concentration of fatty acid (group or individual) at a given time (μg/g dry sed); \( C_0 \) = the original concentration at the start (μg/g dry sed); \( k \) = rate constant (day⁻¹); and \( t \) = incubation time (days).

During an incubation period of 92 days, approximately 40% of solvent-extractable total fatty acids were lost, while there was no significant decrease in the bound fatty acid concentration (Fig. 6). The different fatty acids in the solvent extractable pool degraded at different rates (Table 3). For example, the short-chain fatty acid, 14:0, degraded more rapidly (\( k = 2.2 \) year⁻¹) than the long-chain fatty acid, 24:0 (\( k = 1.09 \) year⁻¹) (Fig. 7a and b). The monounsaturated fatty acid, 16:1, degraded more rapidly (\( k = 2.6 \) year⁻¹) than the saturated fatty acid, 16:0 (\( k = 1.5 \) year⁻¹) (Fig. 7c and d). Polysaturated fatty acid concentrations were very low in these sediments, and did not significantly decrease during the 3 month incubation.
Fig. 4. Changes in solvent-extractable and bound fatty acid subgroup concentrations in the surface sediments (0–2 cm) at seven stations in the Altamaha River: (a) long-chain saturated fatty acids (LCFA); (b) polyunsaturated fatty acids (PUFA); (c) mono-unsaturated fatty acids (MUFA); (d) short-chain saturated fatty acids (SCFA); and (e) branched-chain fatty acids (BCFA).
Table 2
Kinetic parameters (maximum rate and Michaelis constant) of lipase enzymatic reaction at seven stations in the Altamaha estuary

<table>
<thead>
<tr>
<th>Station</th>
<th>1N</th>
<th>2N</th>
<th>3N</th>
<th>4N</th>
<th>5N</th>
<th>6N</th>
<th>7N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$ (nM)</td>
<td>27.3</td>
<td>25.2</td>
<td>18.0</td>
<td>67.8</td>
<td>32.3</td>
<td>28.2</td>
<td>45.5</td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>117</td>
<td>159</td>
<td>134</td>
<td>331</td>
<td>222</td>
<td>150</td>
<td>264</td>
</tr>
</tbody>
</table>

3.5. Changes in lipase activities during sediment incubations

The incubation system consisted of a thin layer (~0.5 cm) of bulk sediment and ~200 ml overlying water to simulate the sediment–water interface and allow solute exchange between two phases. Without overlying water, the lipase activity in bulk sediments was lost within 2 weeks (Fig. 2). During a 3 month incubation period, where overlying water was present, sediment lipase activity increased slightly over time (Fig. 8).

4. Discussion

4.1. Relationship between lipase activity and fatty acid distribution

The mixing at the land–sea margin of the Georgia coast is driven primarily by tides and freshwater runoff (Blanton and Atkinson, 1978). Due to its high discharge, the Altamaha River mixing zone is partially-mixed and the freshwater residence time is less than 1 week (J. Blanton, pers. comm.). The salinity in the Altamaha mixing zone, e.g. between stations 4N and 5N, varies from 0 to 20% during a tidal cycle (Cai and Wang, 1998). Intensive mixing occurs at the salt wedge associated with the salinity front where there are the highest suspended sediment concentrations (Zheng et al., 1999). Intensive mixing causes a rapid deposition of suspended and adsorbed organic matter, which results in a high organic carbon content at stations within the strong mixing zones. Based on the high sediment organic carbon at station 4N (Table 1), it appears that intensive mixing in Altamaha River occurs near this station. Total fatty acid concentrations in Altamaha sediments (Fig. 3) is correlated with organic carbon content, indicating that the increased amount of lipid deposition at station 4N was likely due to intensive mixing.

Organic matter in Altamaha estuarine sediments comes from a variety of sources, including terrestrial vascular plants, plankton produced in the riverine and coastal waters, marsh plants, and benthic organisms. Based on lipid analysis and stable isotope ratios of fatty acids for three cores collected during March 1997 (Shi, 1998), it was shown that particulate lipids from different sources were separately deposited in the Altamaha River mixing zone. Deposition patterns of fatty acids from different sources were also found in different surface sediments in this study. Highest concentrations of long-chain saturated fatty acids (predominately the bound form) in surface sediments were found at station 4N with only low concentrations at the other six sites (Fig. 4a), indicating that primarily terrestrial organic matter was deposited at station 4N. In contrast, highest polyunsaturated fatty acid concentrations (dominated by unbound form) were found near the river mouth (stations 6N and 7N; Fig. 4b), presumably due to input of marine algae. Other subgroups of fatty acids, e.g. monounsaturated, short-chain saturated and branched-chain, had high concentrations at stations 6N, 7N and 4N. The high monounsaturated and short-chain saturated fatty acid concentrations at station 4N was likely due to physical turbulence during tidal cycles. Repeated resuspension/settling of surface particles moves particulate lipids laterally along the mixing zone. Relatively stable monounsaturated and short-chain fatty acids remain in these moving particles while polyunsaturated fatty acids rapidly degrade during transport. During resuspension/settling cycles, bacteria appear to be more resistant to abrasion and death than Eukarya (Findlay et al., 1991), so that branched-chain bacteria fatty acids are distributed along the mixing zone in a pattern similar to monounsaturated and short-chain fatty acids, rather than the patterns found for long-chain saturates and polyunsaturates.

Various fatty acid subgroups showed different relationships with lipase activities (maximum rate) (Fig. 5). No correlation was found between lipase $V_{\text{max}}$ and concentrations of long-chain saturates or polyunsaturates, which implies that sediment lipase activities were not related to fatty acid inputs from terrestrial higher plants or marine algae. The inputs of these two fatty acid subgroups into the sediments varied at the various sites, but lipase activities did not correlate with these different inputs. While enzyme activity is strongly affected by some organic inputs, e.g. phytoplankton production, in some environments (Reichardt, 1986; Meyer-Reil, 1987; Gajewski and Chrost, 1995), no simple relationship has been found between particulate organic carbon and various enzyme activities. For example, seasonal variations in extracellular protease activity were not related to variations in substrate concentrations but were correlated with seasonal temperature changes (Mayer, 1989).

There was a correlation between the monounsaturated, short-chain, and branched-chain fatty acid concentrations and lipase activity (Fig. 5) suggests that sediment lipases have a microbial origin. As noted above, the monounsaturated and short-chain fatty acids in the surface sediments of...
Lipase activity assays showed a variation of ±5% (n = 4).
the mixing zone may be significantly affected by physical turbulence produced by tidal changes. Surface sediments are moved laterally during the resuspension/settling cycle. Resuspension of sediment increases microbial activities in coastal ecosystems (Chrost and Riemann, 1994). The similarity of distribution patterns between branched-chain fatty acids (specific to bacteria) and monounsaturated and short-chain fatty acids in the mixing zone (Fig. 4c–e) implies that microbial processes are enhanced by the physical turbulence produced by tidal changes.

4.2. Fatty acid degradation and lipase activity

The rates and pathways of labile organic matter degradation significantly affect organic carbon cycling in estuarine sediments. Earlier work on sediment lipids found that biologically active sediments contained high fatty acid concentrations, but the ratio of total fatty acid concentrations to total organic carbon in surface sediments was only one tenth that of plankton (Parker and Leo, 1965; Kvenvolden, 1967; Parker, 1967). This implied that fatty acids degraded more rapidly than other sediment organics. Depending on environmental conditions, fatty acids degrade at different rates in different types of sediments (e.g. Farrington et al., 1977; McCaffrey, 1990; Sun and Wakeham, 1994; Cauvel and Martens, 1996; Sun et al., 1997). In Altamaha estuarine sediments, fatty acids can come from different sources as various esters or complexes in solvent-extractable or bound forms. Fatty acids in different esters, e.g. triacylglycerols and phospholipids, and in different sediment types may differ in their reactivity with lipase. Laboratory sediment incubations in this study provided information on reactivity of different subclasses of fatty acids with lipase activity.

There were significant differences in reactivity between solvent-extractable and bound fatty acids (Fig. 6). Solvent-extractable fatty acids degraded continuously with approximately 40% degraded after 3 months, while bound fatty acids were not significantly degraded during this same time period. The high degradation rate constants of solvent-extractable fatty acids can be related to their source, which is assumed to be fresh organic material. Solvent-extractable lipids were higher in concentration than bound lipids in surface sediments during a phytoplankton bloom (Sun and Wakeham, 1999). In the Altamaha estuarine sediments, algal-derived polyunsaturated fatty acids were primarily present in the solvent-extractable form. Bound (saponification released) fatty acids are tightly bound to the sediment matrix, and it is assumed that this binding takes place long after the original input. Long-chain saturated fatty acids, derived from terrestrial plants, were the dominant fatty acid subgroup bound to the sediments. Bound long-chain saturated fatty acids are slowly degraded while they are transported downstream over a relatively long time.
scale, i.e. years. Higher degradation rate constants of solvent-extractable fatty acids suggest that lipid-degrading enzymes, e.g. lipase, hydrolyze fatty acids from esters and complexes in recent organic materials prior to degradation of the released fatty acids. In contrast, fatty acids tightly bound to the sediment matrix are probably inaccessible to the lipase, thus resulting in a lack of bound lipid degradation during 3 months of incubation.

For solvent-extractable fatty acids, structural effects strongly affected fatty acid degradation rates. Unsaturation (presence of double bonds) was an important factor controlling lability of fatty acids: unsaturated 16:1 degraded faster than saturated 16:0, which is consistent with laboratory (Harvey and Macko, 1997; Sun et al., 1997) and field studies (Farrington et al., 1977; Sun and Wakeham, 1994; Canuel and Martens, 1996). Chain length also affected fatty acid lability. The degradation rate constant of 14:0 was twice that of 24:0 (Table 3). The pattern of fatty acid chain lengths in sediment was linked to the source, e.g. terrestrial higher plants vs. algae. Other studies found rapid short-chain fatty acid degradation and slow long-chain fatty acid degradation in shallow coastal sediments (Haddad et al., 1992), settling particles in Lake Michigan (Meyers and Eadie, 1993), and deep sea sediments (de Barr et al., 1983).

There are a number of sources for extracellular enzymes in aquatic systems. Besides being produced by living bacteria, they are released during lysis of dead and decaying cells (Corpe and Winters, 1972). Higher extracellular enzyme activities found during phytoplankton blooms could be the result of extracellular enzymes that are released after zooplankton grazing (Gajewski and Chrost, 1995). Lipase activity has been shown to be associated with the bacterial size fraction of seston (Gajewski et al., 1993). However, Mayer (1989) found a lack of correlation between enzyme activities and either substrate or bacterial concentrations in sediments. In the present study, lipase activities increased slowly during the decrease in solvent-extractable fatty acids in a 3 month incubation study, suggesting that lipase activities were not dependent on substrate concentrations. Overlying water needed to be present for

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**Fig. 7. Changes in solvent-extractable fatty acid concentrations during a 3 month incubation of sediment from station 5N: (a) 14:0; (b) 24:0; (c) 16:1; and (d) 16:0.**

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this lipase activity to increase. In the absence of over-
lying water, lipase activities rapidly decreased (Fig. 2).
The sediment–water interface is an active diagentic zone
associated with an active microbial community (Klump
and Martens, 1981; Deming and Baross, 1993). It seems
likely that the increased lipase activity was due to
microbial activity increases at the water–sediment inter-
face.

5. Conclusions

Fatty acid distribution patterns in Altamaha estuar-
ine sediments showed that the deposition of particulate
organic matter derived from terrestrial vascular plants
and marine algae is decoupled. Sediment lipase activities
related differently to the various fatty acid subgroups.
No correlation was found between lipase activity and
long-chain saturated (terrestrial plants) and poly-
unsaturated (algae) fatty acids concentrations. Lipase
activities did correlate with monounsaturated, short-
chain, and branched-chain (bacteria specific) fatty acids.
Thus, the lipase was likely produced by bacteria. Dif-
ferences in the reactivities between solvent-extractable
fatty acids and bound fatty acids may be due to differ-
ences in the accessibility of lipase to natural matrixes.
Solvent-extractable fatty acids, associated with fresh
organic matter, can be readily released from their esters
and the released fatty acids further degraded, while
lipids tightly bound to the sediment may be inaccessible
to lipases. Structure effects, e.g. degree of unsaturation
and chain length, are also important factors affecting
fatty acid degradation rates in Altamaha estuarine sedi-
ments. Lipase activity increases during a 3 month incu-
bation, were presumably due to stimulation of microbial
activities at the sediment–water interface.

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Fig. 8. Lipase activity changes during a 3 month incubation of
station 5N sediment.
