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Degradation of algal lipids in microcosm sediments with different mixing regimes

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Abstract

A series of microcosm experiments was conducted to examine the influence of different mixing processes on algal lipid degradation in surficial sediments. The experiments simulated 3 different mixing regimes: bioturbated, episodically physically mixed, and no mixing. Uniformly ^{13}C- and ^{15}N-labeled algal cells were deposited on the sediment-water interface to simulate natural deposition of bloom-produced organic matter. Degradation rate constants of individual algal lipids, effects of macrofuana species and physical mixing frequency on these rates, and the response of the microbial community were examined during one-month incubations. Our results showed that different mixing processes established different biogeochemical degradation regimes, characterized by variable redox conditions. Algal lipids degraded differently in different mixing regimes. Slower degradation was observed under episodic physical mixing because substrates were mixed deep into the sediment where anaerobic conditions prevailed. The degradation rates of algal lipids subjected to oscillating redox conditions during bioturbation were similar to those in unmixed cores where aerobic conditions dominate at the surface. Our laboratory results support the OET (oxygen exposure time) model based on field observations: longer residence of organic matter in the oxic zone leads to low preservation. Biological and physical mixing processes in surface sediments affect degradation and preservation of organic matter through different controls on redox conditions.

1. Introduction

In shallow coastal regions, algal blooms result from natural and anthropogenic variations in nutrient loading and stratification. Blooms deliver large amounts of fresh organic matter to the sediment-water interface, where benthic organisms degrade the labile organic compounds in the deposited material. Decomposing organic matter is a driving force for biogeochemical reactions occurring in surficial sediments (Berner, 1980). The rate and extent of organic matter degradation significantly affects carbon burial, nutrient cycling, the abundance and activity of benthic organisms, metal redox chemistry, and the fate of contaminates (Cowie and Hedges, 1994; Keil et al., 1994; Hedges and Keil, 1995; Sun et al., 1999).

Organic matter degradation in surficial sediments occurs by either aerobic or anaerobic pathways, depending on in-situ oxygen concentrations. After O_2 is consumed, series of electron acceptors (i.e., NO_3^-, Mn and Fe oxides, SO_4^{2-}, and CO_2) are used subsequently by bacteria to decompose organic compounds in a sequence that depends on the yield of metabolic free energy (Froelich et al., 1979). In coastal marine sediments, oxygen and sulfate are mostly responsible for the bulk of carbon remineralization (Jørgensen, 1983; Mackin and Swider, 1989). However, recent work has demonstrated that intermediate oxidants (NO_3^-, Mn and Fe oxides) can play a significant role in remineralization of organic matter in organic-rich coastal sediments

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(Canfield et al., 1993; Aller, 1994a). At present, our understanding of, and ability to model the effects of aerobic vs. anaerobic conditions on organic matter degradation is limited, especially at the level of sediment microstructure and specific organic molecules (Emerson and Hedges, 1988; Pedersen and Calvert, 1990; Lee, 1992; Canfield, 1994).

The spatial distribution of aerobic and anaerobic conditions in coastal sediments is strongly influenced by various mixing processes. Biological mixing (bioturbation) is carried out by benthic macrofauna which alter aerobic/anaerobic boundaries, fragment particles, regulate material transport, and directly consume, digest and degrade organic matter. As a result of bioturbation, organic particles are mixed continuously through the entire spectrum of sediment redox conditions (Aller, 1982). The frequency and duration of mixing influences organic matter degradation (Sun et al., 1993; Aller, 1994b). In sediments that are subjected to episodic physical mixing, for instance during severe storms, both particulate organic matter and dissolved oxygen can be mixed deep into sediments which were previously anaerobic. Physical mixing processes may involve erosion, sediment transport, and burial of fresh organic matter in deep anaerobic sediments during redeposition. In contrast to bioturbated sediments, fresh organic matter buried during strong physical mixing is not mixed back into the aerobic zone and is degraded by anaerobic pathways.

This study was designed to examine the effects of different mixing processes on degradation of phytoplankton lipids. Three mixing regimes: bioturbated, episodically physically mixed, and no mixing were simulated in laboratory microcosms. Uniformly ^{13}C- and ^{15}N-labeled algae were added into the microcosms to simulate sedimentation of bloom-derived organic matter. We monitored selected algal lipids as proxies for fresh organic matter because of several considerations: (1) lipids are one of three major classes of organic matter in algal material (~5–20%, Parsons et al., 1961); (2) they are less reactive than proteins and carbohydrates and thus are used as biomarkers in preserved organic matter (Volkman et al., 1987; Wakeham and Lee, 1993); (3) they are sensitive to redox conditions and degrade at different rates in different depositional environments (McCaffery et al., 1991; Sun and Wakeham, 1994; Canuel and Martens, 1996; Sun et al., 1997; Harvey et al., 1997a); and (4) specific lipids have been widely used to characterize bacterial communities (Dowling et al., 1986; Findlay et al., 1985; Vestal and White, 1989). Changes in the inventory of labeled lipids with time allowed us to estimate lipid degradation rate constants in different mixing regimes. We also assessed the microbial response by tracking the production of labeled bacteria-specific compounds produced during incubations. The effects of macrofauna species and frequency of physical mixing on algal lipid degradation were specifically examined in detail. We focus only on the degradation of algal lipids in this study; other data will be reported elsewhere.

2. Experimental

2.1. Materials

Sediment, water samples, and benthic animals used in this study were collected from a site in Doboy Sound (31°23.52′N, 81°17.64′W) near Sapelo Island, Georgia, USA. The sediment consists of approximately 40% silt and 60% fine sand. Organic carbon content of the sediment is typically 1–2% by weight. Benthic macrofauna at this site are dominated by three major taxa (Polychaeta, Mollusca and Crustacea), which is similar to the macrofauna at nearby locations off Sapelo Island (Leiper, 1973; Pinet and Frey, 1977; Tenore, 1985). The abundance and species of macrofauna in this area vary seasonally over a large range (500–25,000/m^2 of total number individuals and 50–100 of species, Leiper, 1973). Seawater (salinity ~28‰) used in the incubations was collected by pumping through a set of filters (25 μm). Sediment was collected using a box corer (surface area ~0.04 m^2). The top 1 cm of sediment was scraped from the box core for use in the experiments. Benthic macrofauna were obtained by sieving the top a few cm of sediment through a 1-mm sieve (with water). Macrofauna were carefully removed and placed in a container of water with a thin layer of sediment at the bottom. The container was oxygenated by an aquarium air pump during transport to our laboratory (~5 h). Surficial sediment was held at the in situ temperature for a few days and then passed though a 0.25-mm sieve (no water) to remove macrobenthos, large shells and detritus. Benthic animals were separated by species (identification with help from Dr. D. Bishop) prior to setting up experiment. Dual-labeled algae (*Chlorella*, $^{13}C > 98\%$ and ^{15}N ~96–99%), obtained from Cambridge Isotope Laboratories, were used as the tracer. The major lipids of this alga were uniformly ^{13}C-labeled fatty acids [16:1(ω7), 16:0 and 18:1(ω9)] and phytol, hence they were the target compounds for this study.

2.2. Microcosm setup

Experiments were conducted in microcosms constructed from sections of core liner (i.d. 7 cm). The bottom of these sections were sealed with caps, then they were filled with sieved surficial sediment to a depth of ~15 cm. Labeled algae and glass beads (~100 μm) as a particle tracer were mixed with sieved surficial sediment (~60 mg of labeled algae and ~40 mg of glass beads in ~14 g wet sediment) and then fashioned into thin (1 mm) sediment cakes with the same diameter as the

cores. The addition of labeled algal cakes was designed to simulate the natural pulse of phytoplankton material that settles to the bottom after a water column bloom. After freezing overnight, these sediment cakes were placed on the sediment surface in microcosms. Subsequently, ~500 ml of filtered seawater was gently added to the microcosm. The overlying seawater in all microcosms was continuously purged with air so that it remained oxygenated during incubations. After preparing different treatments (adding animals, physical stirring, and no mixing), all microcosms were placed in a large reservoir with enough seawater to equal the water height in the cores. The microcosms were then incubated in a temperature-controlled incubator in the dark.

Three experiments were conducted during March 99 (Exp. A) and July 99 (Exp. B and Exp. C). Exp. A used 5 replicates of each of three treatments: (1) unmixed control cores (no macrofauna and no stirring); (2) bioturbated [5 *Paraprionospia pinnata* (spionid polychaeta), 1 *Ilyanassa obsoleta* (gastropod mollusca), and 1 *Mulinia lateralis* (pelecyopod mollusca) added to each core]; (3) physically mixed (mechanically stirred once to 5 cm depth for 2 min at the beginning of the experiment). The animals used in Exp. A were the dominant species present at the sampling site during spring. One microcosm from each treatment was sacrificed for lipid analysis at intervals during the course of the one-month incubation. In Exp. B, eight microcosms were prepared as follows: (1) control (4 microcosms); (2) 5 *Paraprionospia pinnata* in each of 2 microcosms; (3) 6 *Ogyrides limnicola* (decopod crustacea) in each of 2 microcosms. These animals were the dominant macrofauna encountered at the study site during summer. Two control microcosms (duplicates) were sacrificed immediately to establish initial conditions ($t=0$ days) and the remainders were sampled after incubation as above for 28 days. In Exp. C, four sets of microcosms were set up as follows: one set (two initially stirred cores) were sacrificed immediately and the other three sets (two cores in each set) were incubated for 29 days with physical mixing at different frequencies (once each 3, 6, and 12 days, respectively). Overlying seawater in each microcosm in all incubations was changed weekly to avoid excessive accumulation of metabolites such as NH_4^+. At the end of one-month incubations, most macrofauna were observed to be alive although they were not separated from the sediment.

2.3. Sampling and biogeochemical analyses

On the sampling day, we first measured the profiles of oxygen, pH and pCO_2 using microelectrodes following the method of Cai et al. (2000), then the overlying seawater was removed and filtered for chemical analyses. Sediment was extruded from the microcosm and sliced into 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5, 5–7, and >7 cm depth intervals. About 0.5 g of wet sediment from each depth was dried at 60 °C for 24 h to estimate the porosity. Dried sediments were gently ground and ~40 mg of the sediment was mixed with water for counting glass beads under the microscope. Another 0.2–0.3 g wet sediment was treated with 10% HCl to remove carbonate, lyophilized, then POC, PON, ^{13}C and ^{15}N isotope ratios were determined using a Finnigan Delta C Stable Isotope Mass Spectrometer coupled to a Carlo Erba CHN analyzer. Approximately 2 ml of wet sediment was frozen immediately for subsequent determination of bacteria abundance and for analysis of bacterial community structure. All remaining sediment was centrifuged at 2500 *g* to separate pore water from bulk sediment. The sediment was frozen for lipid analysis while NH_4^+, NO_2^-, alkalinity, DIC and carbon and nitrogen isotope ratios were measured in pore water and in samples of overlying water.

2.4. Extraction and analysis of lipids

Detailed procedures for extraction and analysis of sedimentary lipids are described in Sun et al. (1997; 2000). Briefly, ~1–2 g of thawed sediment was extracted with 10 ml methanol, followed by 3×10 ml extraction with methylene chloride-methanol (2:1 v/v); samples were sonicated for 6 min during each extraction. All extracts were combined and dissolved lipids were partitioned into a methylene chloride phase formed by addition of 5% NaCl solution. The methylene chloride phase was removed and reduced to 1 ml via rotary evaporation. Lipids were saponified using 0.5 M KOH in $MeOH/H_2O$; neutral lipids were extracted from the basic solution (pH > 13) while acidic lipids were extracted following addition of HCl (pH < 2). Fatty acids in the acidic lipid extract were converted to fatty acid methyl esters (FAMEs) by reaction with BF_3^- MeOH at 100 °C for 2 h. Phytol and other alcohols in neutral lipid extracts were treated with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile to form their TMS(trimethylsilyl)-ethers. FAMEs and alcohol-TMS ethers were analyzed by capillary gas chromatography using a Hewlett-Packard 6890 GC. 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, then 150–310 °C at 4 °C/min and finally isothermal at 310 °C for 5 min. 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. Generally, the sample standard deviations were ±3% for various fatty acids and ±5.8% for alcohols, based on duplicate measurements.

3. Results

3.1. Variations of oxygen penetration during various incubations

The depth of dissolved oxygen penetration into sediments and changes in the position of the aerobic/anaerobic boundary differed in microcosms with different treatments (Fig. 1). In Exp. A, dissolved oxygen penetrated to a depth of 2 mm in control (unmixed) microcosms and varied little during the one month incubation. In contrast, dissolved oxygen penetrated deeper (to ~5 mm) in microcosms containing animals. Dissolved oxygen was initially higher at depth in physically mixed microcosms, but the aerobic/anaerobic boundary had migrated to the same depth as in the control microcosms after one month.

In Exp. B, dissolved oxygen penetration was similar (~2 mm) in unmixed and *Paraprionospia* bioturbated microcosms, while the aerobic/anaerobic boundary was markedly deeper (~5 mm) in microcosms containing *Ogyrides*. In Exp. C, oxygen penetration was greater only in microcosms with frequent stirring (once every 3 days) after a 29 d incubation.

3.2. Sediment mixing

The vertical distribution of glass beads in various treatments was different (Fig. 2). In Exp. A, glass beads were immediately mixed into the sediment by stirring, while there was little vertical movement of glass beads in either unmixed or bioturbated microcosms. In Exp. B, glass beads were mixed into the sediment only in microcosms with *Ogyrides*. In these treatments, the maximum concentration of glass beads was at 1–2 cm depth, while most glass beads in unmixed and *Paraprionospia* microcosms remained at the surface (0–0.5 cm) after four weeks of incubation. In Exp. C, glass beads were similarly distributed with depth in all microcosms, regardless of the frequency of physical mixing.

3.3. Consumption of ^{13}C-labeled algal lipids during incubations

The vertical distributions of ^{13}C-labeled algal lipids [16:1(ω7), 16:0, 18:1(ω9), and phytol] were affected by both degradation and transport processes (Fig. 3). Initially ($t=0$), all ^{13}C-labeled algal lipids were at the sediment-water interface. After a one-month incubation, algal lipid concentrations were dramatically lower in the top sediments (0–0.5 cm) of all microcosms. In Exp. A (top panel in Fig. 3), algal lipids had similar vertical distributions in control and bioturbated microcosms, that is, most remaining ^{13}C-labeled lipids still remained in the top 0–0.5 cm. In contrast, a significant amount of ^{13}C-labeled lipid was immediately transported to depth in the physically mixed microcosms and remained there during the one-month incubation.

In order to compare the relative loss rates of algal lipids in different treatments, we integrated measured lipid concentrations over the entire depth of the microcosm. In Exp. A, lipid inventories decreased faster in control and bioturbated microcosms than in physically mixed microcosms (Fig. 4, top and middle panels). In Exp. B, algal lipid degradation varied by individual compounds and by different mixing regimes (Table 1). Most of the remaining ^{13}C-labeled algal lipids were found in the top 0–0.5 cm of control microcosms after four weeks (middle panel in Fig. 3). Some of the remaining ^{13}C-labeled algal lipids were mixed down 2–3 cm in the animal treatments, this effect being more pronounced in the *Ogyrides* microcosms than in *Paraprionospia* microcosms (middle panel in Fig. 3). ^{13}C-labeled 16:1 fatty acid was completely degraded in *Ogyrides* microcosms, although other lipids persisted in these microcosms. In all treatments of Exp. C, inventories of ^{13}C-labeled unsaturated fatty acids (16:1 and 18:1) varied less than saturated 16:0 fatty acid and neutral phytol (Table 1). Regardless of the stirring frequency in these microcosms, individual ^{13}C-labeled algal lipids existed at similar concentration over the entire mixed depth (5–6 cm).

3.4. Production of new ^{13}C-labeled lipid compounds

Several new ^{13}C-labeled lipid compounds including 14:0, *iso*-15:0, *anteiso*-15:0, 15:0 and 17:0 fatty acids and one normal C_{16} alcohol were produced during the incubations. These ^{13}C-labeled lipids were distinguished from their natural (^{12}C) counterparts by the relative ratios of the characteristic fragments detected in mass spectral analysis (Sun, 2000). None of these ^{13}C-labeled compounds were present initially, but their concentrations increased appreciably during the incubations (Fig. 5 and Table 1). In Exp. A, most newly-formed ^{13}C lipids were found in the top 0–0.5 cm of control and animal treatments. For physically mixed microcosms, new ^{13}C-labeled fatty acids were observed throughout the mixed depth. In control and *Paraprionospio*-containing microcosms of Exp. B, most newly-formed ^{13}C-labeled fatty acids occurred in surficial sediments, however, they were observed at depth (down to 2–3 cm) in microcosms containing *Ogyrides*. The ^{13}C-labeled C_{16} alcohol was detected only in control and *Paraprionospio*-containing microcosms, but not in *Ogyrides*-containing microcosms. In Exp. C, new ^{13}C-labeled fatty acids occurred throughout the entire mixing depth of all physically-mixed microcosms but little of the ^{13}C-labeled C_{16} alcohol was detected. When the concentration profiles of these newly-produced ^{13}C-labeled lipids were integrated, the time-dependent variation in inventory

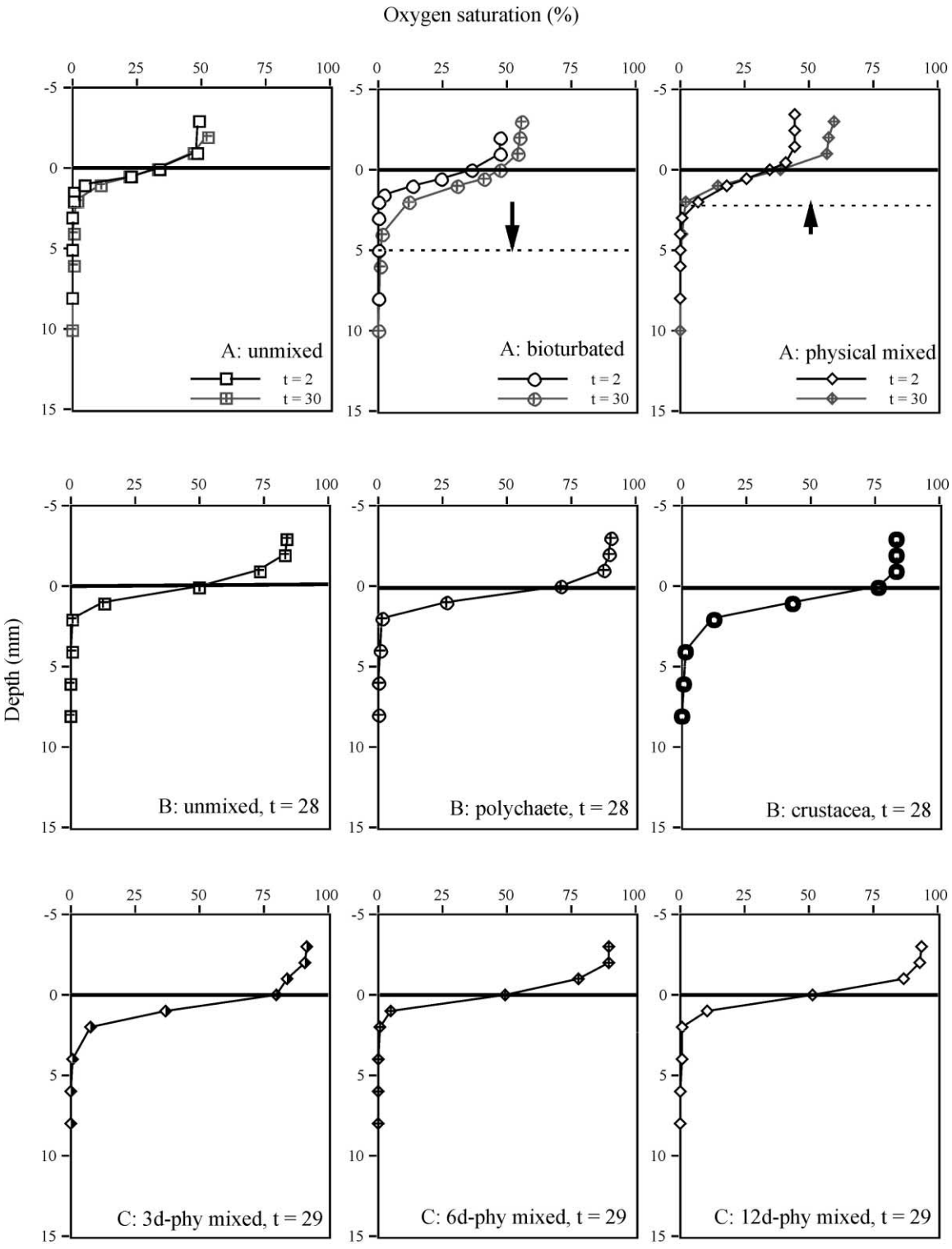

Fig. 1. Dissolved oxygen profiles measured in experiments A (top row), B (middle row), and C (bottom row). Dotted and dashed lines in the top row show oxygen penetration depth. The arrows in the top row show direction the oxic/anoxic boundary moved during Exp. A incubations. Dotted lines in the middle and bottom rows (Exps. B and C) show oxygen penetration depth after 4 weeks of incubations.

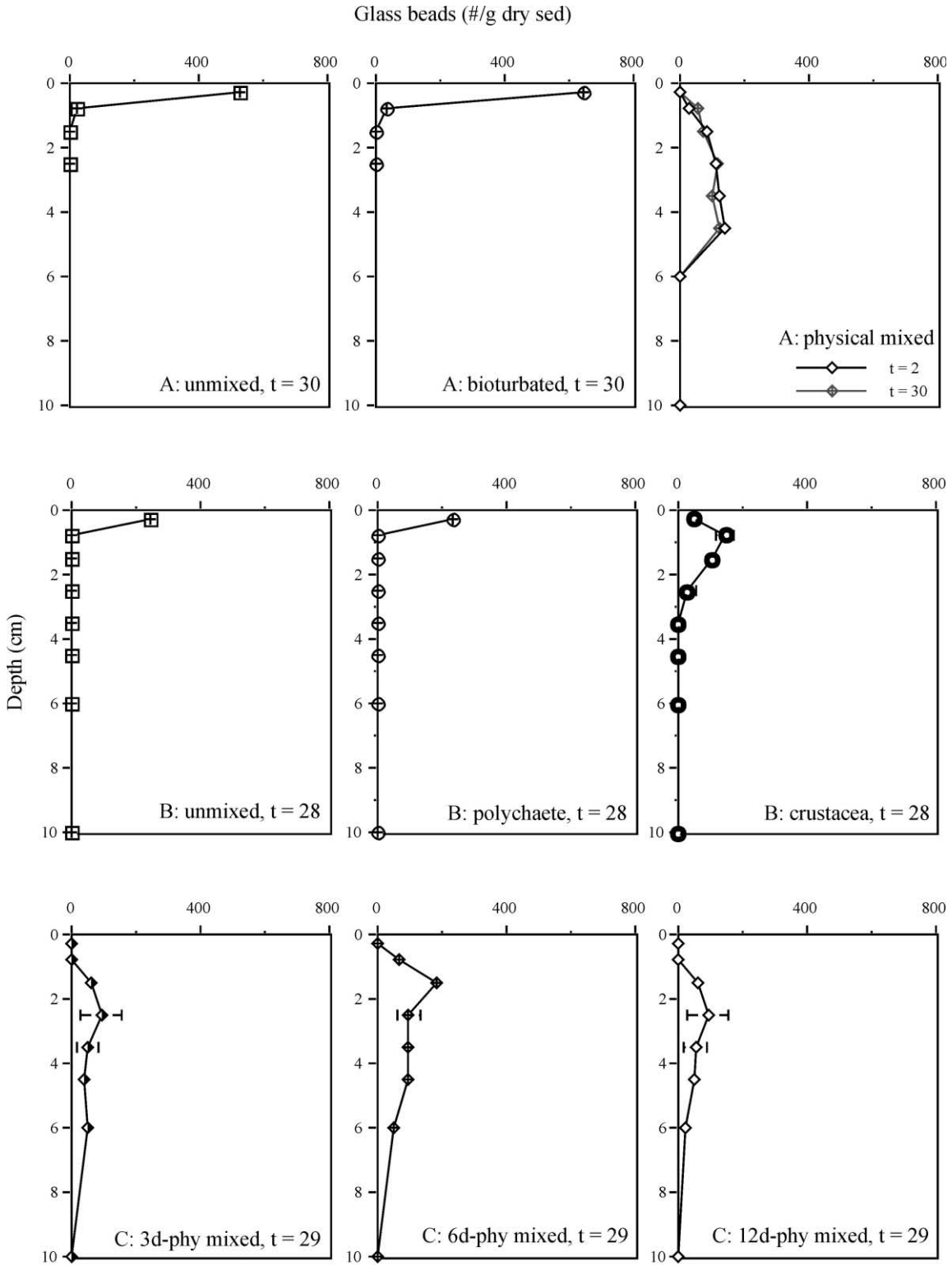

Fig. 2. Final distributions of glass beads in experiments A (top row), B (middle row), and C (bottom row). Error bars in the middle and bottom rows show the relative standard deviation based on duplicate core measurement.

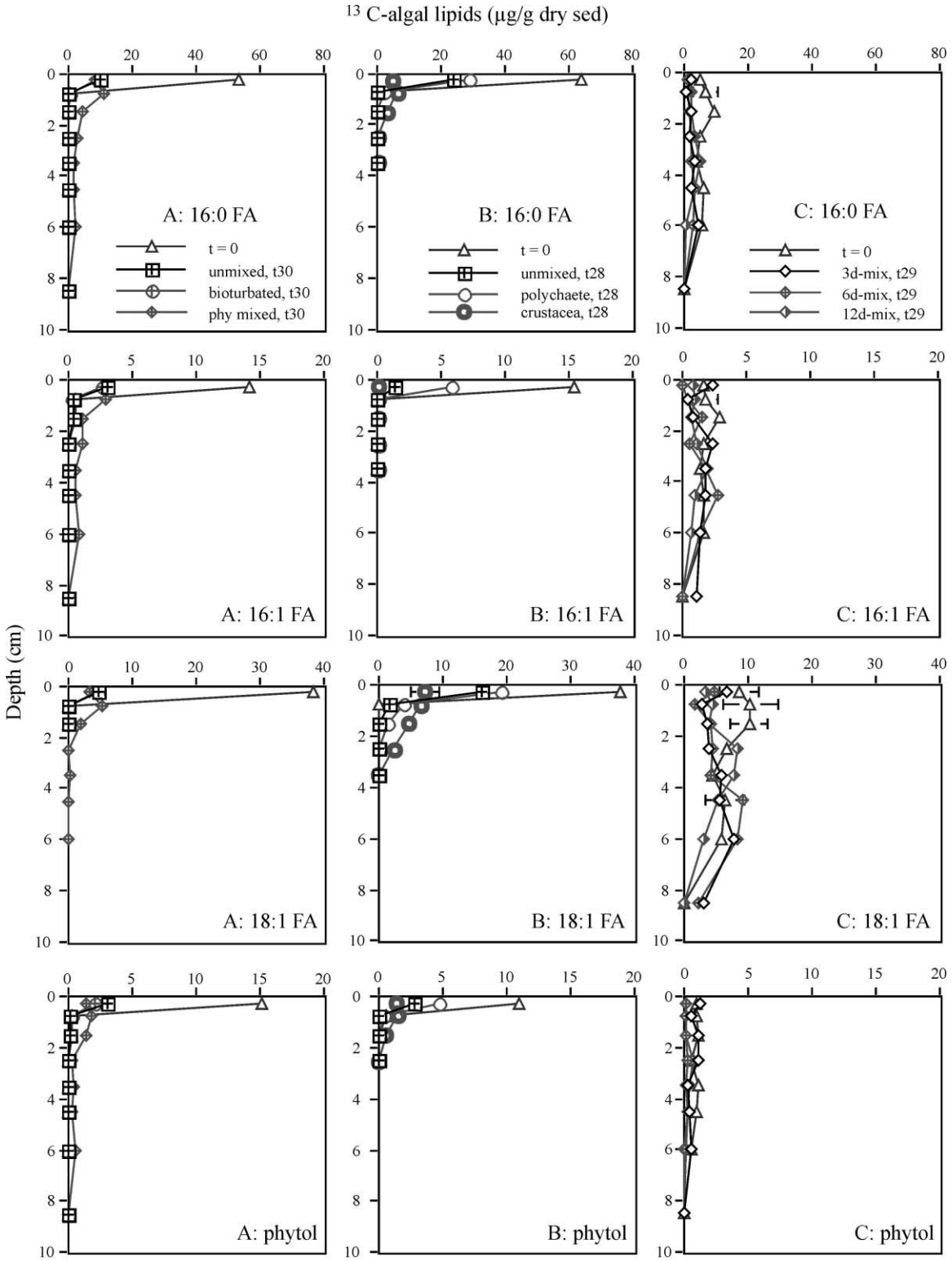

Fig. 3. Profiles of various ^{13}C-labeled algal lipids (16:0, 16:1, 18:1 fatty acids, and phytol) measured at t = 0 and at the end of incubations A (first column), B (second column), and C (third column).

among different mixing regimes (Exp. A) was apparently different (Fig. 4). More new, bacteria-specific ^{13}C-labeled fatty acids accumulated in physically-mixed, than in unmixed microcosms. Although production of the ^{13}C-labeled C_{16} alcohol was initially rapid in all three mixing regimes of Exp. A, the concentration of this compound gradually decreased to a very low level in unmixed and animal treatments during the incubation. In Exps. B and C, there was appreciable production and accumulation of ^{13}C-labeled fatty acids in all treatments (Table 1). However, ^{13}C-labeled C_{16} alcohol was not observed in treatments that were repeatedly physically mixed (Exp. C), nor in *Ogyrides*-containing microcosms (Exp. B).

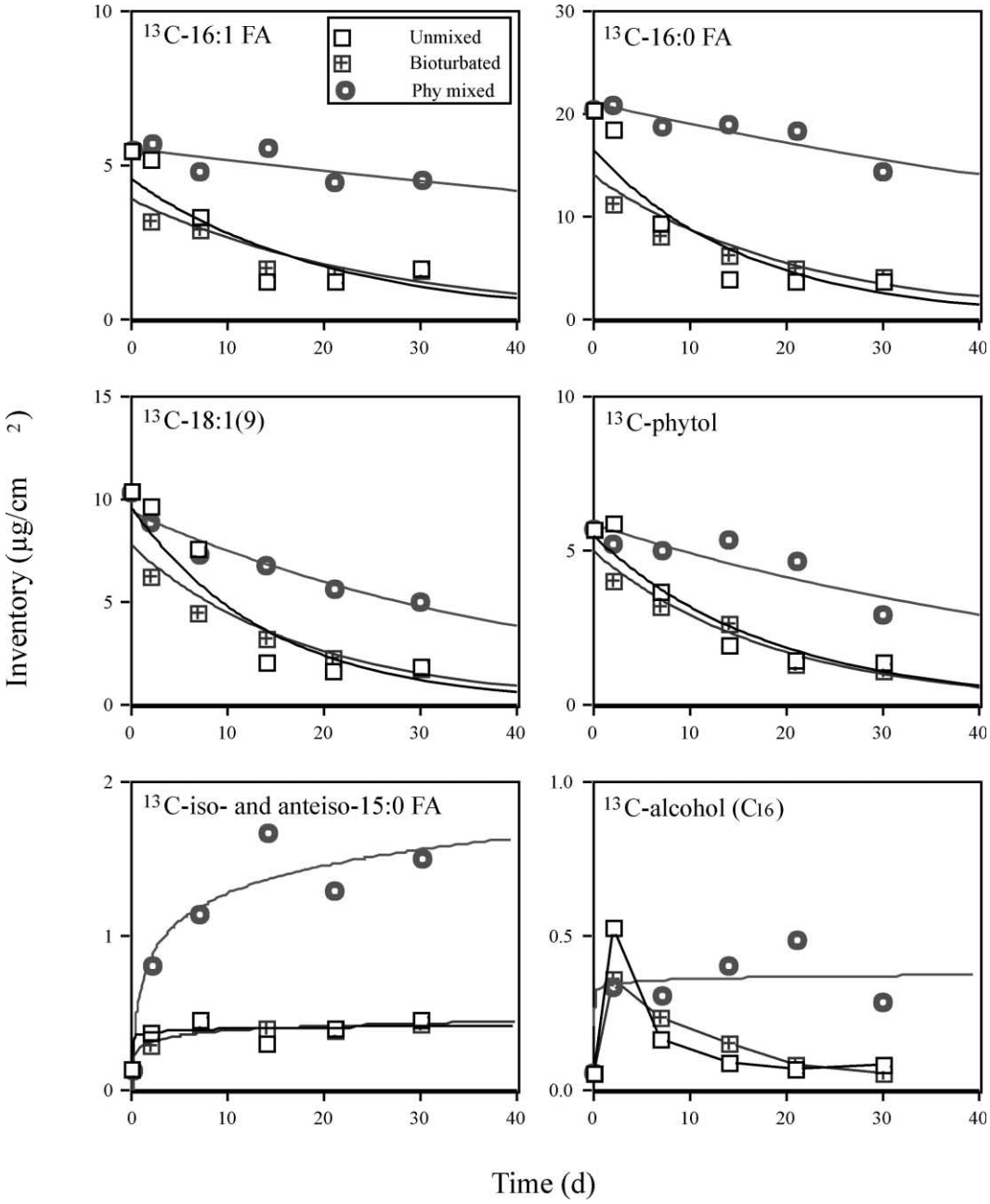

Fig. 4. Time-dependent inventory variations of ^{13}C-labeled algal lipids (16:0, 16:1, 18:1 fatty acids, and phytol) and newly-produced ^{13}C-labeled bacteria-specific fatty acids (iso- and anteiso-15:0 fatty acids) and C_{16} alcohol in experiment A. The solid lines are curves fit to a first-order degradation model of the data and the dashed lines are used only to facilitate viewing.

4. Discussion

4.1. Mixing mechanisms and consequence

In this study, mixing was tested as a major factor affecting degradation and preservation of algal organic matter. Emphasis was placed on a comparison of physical and biological mixing processes. Mechanisms of biological and physical mixing are different for solid and dissolved phases. Benthic macrofauna rework sediments by burrowing, crawling, and feeding, which are collectively described as a continuous biodiffusion process (Berner, 1980; Aller, 1982). Dissolved components are primarily mixed between sediment and overlying water by irrigation. In our Exps. A and B, variations in dissolved oxygen penetration depth were affected by the presence and abundance of different macrofauna. When three species (one polychaete and two molluscs) coexisted in the animal treatments of Exp. A, dissolved oxygen gradually penetrated deeper (from 2 mm to 5 mm, Fig. 1) during the incubation. Deeper penetration probably resulted from the activities of the two molluscs because the polychaete species used in this experiment usually resides in a subsurface layer of the sediment (2–3 cm depth below the surface). Exp. B, where individual macrofauna species were used separately in each treatment, further demonstrated that the polychaetes used in our experiments did not enhance oxygen penetration significantly (Fig. 1). The crustacean used in Exp. B rapidly built burrows and thus facilitated penetration of dissolved oxygen (Fig. 1). In physically stirred microcosms, dissolved oxygen was initially mixed down to 4 mm depth; however, the aerobic/anaerobic boundary gradually returned to a depth that was similar to unmixed microcosms (2 mm). Oxygen penetration was also affected by the frequency of physical stirring (Fig. 1). The aerobic/anaerobic boundary moved down in biologically mixed treatments but upward in physically mixed treatments.

Table 1
Comparison of ^{13}C-labeled lipid (algal and newly-produced) inventories ($\mu g/cm^2$) in sediment microcosms during experiment B (comparison of macrofauna species: two cores with *Paraprionospir pinnata*, two cores with *Ogyrides limnicola*, and four control cores) and experiment C (comparison of physical mixing frequency: two cores with 3 d mixing, two cores with 6 d mixing, two cores with 12 d mixing, and two cores with no mixing). The numbers in parentheses are standard deviations estimated from duplicate core measurements

Exp. B	$t=0$, unmixed	$t=28$, unmixed	$t=28$, polychaete	$t=28$, crustacean
16:0 FA	16.33 (±1.31)	5.77 (±0.37)	6.10 (±0.46)	3.92 (±0.13)
16:1 FA	3.37 (±0.03)	0.32 (±0.13)	1.11 (±0.09)	0.00
18:1 FA	9.92 (±0.45)	5.34 (±0.34)	5.66 (±0.07)	7.22 (±0.35)
Phytol	2.69 (±0.19)	0.71 (±0.26)	1.04 (±0.08)	0.62 (±0.02)
i-15:0 FA	0.01 (±0.004)	0.42 (±0.05)	0.50 (±0.08)	0.73 (±0.07)
a-15:0 FA	0.01 (±0.002)	0.14 (±0.005)	0.21 (±0.045)	0.47 (±0.08)
C_{16}-alcohol	0.00	0.06 (±0.017)	0.09 (±0.007)	0.00
Exp. C	$t=0$, mixed	$t=29$, 3d-mix	$t=29$, 6d-mix	$t=29$, 12d-mix
16:0 FA	17.97 (±2.96)	8.82	8.18	10.10
16:1 FA	4.88 (±0.34)	4.13	4.39	3.55
18:1 FA	9.77 (±2.11)	8.14	10.19	9.65
Phytol	2.05 (±0.06)	1.81	0.77	0.35
i-15:0 FA	0.16 (±0.036)	0.67	0.61	1.00
a-15:0 FA	0.17 (±0.027)	0.55	0.53	0.63
C_{16} alcohol	0.00	0.00	0.00	0.00

The solid phase mixed differently from the dissolved phase in all treatments. In physically mixed treatments, solid phase tracers (nonreactive glass beads) were transported downward and the maximum amount was found at depth 4–5 cm immediately following the initial stirring (Exp. A). With repeated stirring during the incubation (Exp. C), the vertical distributions of glass beads became more uniform (Fig. 2). However, physical stirring caused sorting of particles by their size and density, especially for treatments that were only stirred once (Exp. A). Low density algal material may redistribute with depth differently from glass beads, that is, most of the algal biomass may remain at the surface (0–2 cm) rather than being mixed to the bottom of the mixed layer (4–5 cm).

In bioturbated treatments, mixing of the solid phase seems to be dependent on the animal species. Only the crustacean played a significant role in solid phase redistribution (Fig. 2). Polychaetes and molluscs did not cause notable downcore mixing of the solid phases. The differences in bioturbation between treatments are likely related to the animal's feeding and reworking behaviors. In microcosms containing crustaceans, very fine suspended particles were observed in the overlying water and visible burrows were built in sediments. The crustaceans may preferentially feed on the added labeled algal material at the sediment-water interface. We also noted that crustaceans more efficiently irrigated the surface sediments through their burrows than polychaetes,

resulting in deeper oxygen penetration (Fig. 1). This may be caused by a higher oxygen requirement for crustaceans, which are burrow builder, relative to polychaetes, which are deposit feeders. In polychaete and mollusc microcosms, neither suspended particles nor visible burrows were observed. Polychaetes were found in a sublayer (<2 cm) and one mollusc species (*Ilyanassa obsoleta*) was always observed at the sediment-water interface. *Mulinia lateralis* is a filter feeder and *Ilyanassa obsoleta* is a carnivore, thus these animals may not feed on the fresh algae which were already mixed into the surface (1 mm) sediments.

A major consequence of mixing processes in surficial sediments is to alter redox conditions, since organic matter may degrade differently in aerobic and anaerobic zones. Physical mixing simulated in this study initially caused downward transport of dissolved and solid components. The redox boundary (controlled by dissolved oxygen penetration) gradually returned the same depth as in unmixed treatments, but solid organic particles remained buried in anaerobic sediments. Under strong biological mixing (e.g., in the crustacean treatment), organic particles were continuously moved up and down; they oscillated between aerobic and anaerobic

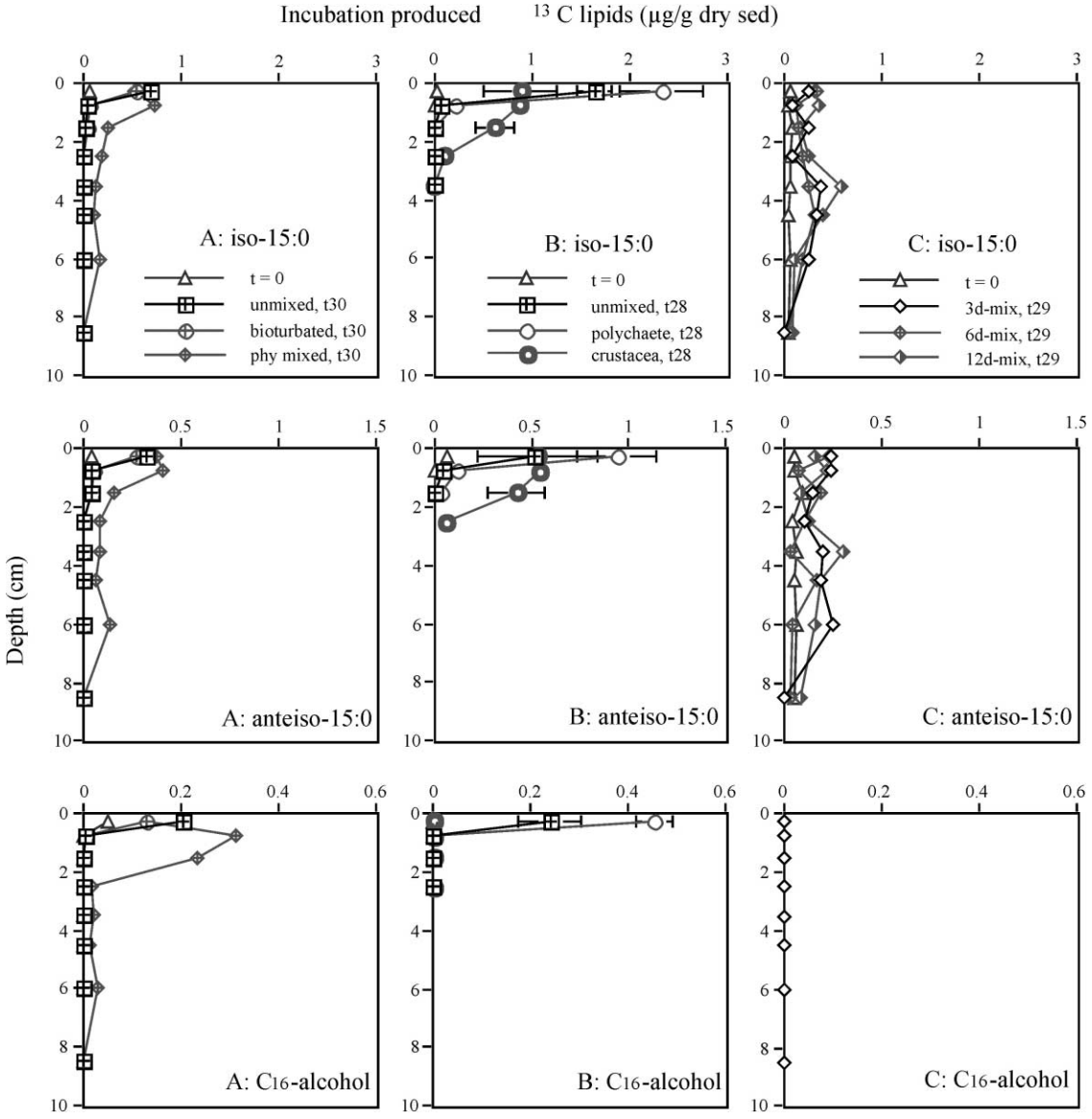

Fig. 5. Profile comparison of various newly-produced ^{13}C-labeled lipids (iso-15:0, anteiso-15:0, and C_{16} alcohol) measured at $t = 0$ and at the end of incubations A (30 d), B (28 d), and C (29 d).

zones as a result of the animal's activity. Thus, in our incubations, ^{13}C-labeled algal material was degraded through several pathways: aerobic (in unmixed microcosms), anaerobic (at depth in physically mixed microcosms), and under oscillated aerobic/anaerobic conditions (in bioturbated microcosms).

4.2. Degradation of algal lipids in different mixing regimes

The vertical distribution and degradation rates of algal lipids in the experimental microcosms are significantly affected by various mixing processes (Figs. 3–4). Since the labeled algal lipids initially added at the sediment-water interface can be simultaneously degraded and transported downward, the loss of labeled algal lipids from the interface should be better quantified by following variation of lipid inventory throughout the entire mixed depth (Sun et al., 1999). Overall lipid degradation was quantified using a first-order decomposition kinetic model that assumes a logarithmic decrease in lipid inventory with time ($dI_i/dt = -k_i I_i$, where I_i = inventory of lipid compound i). Decomposition rate constants were calculated from the slopes of log-transformed lipid inventories versus time in the entire mixed zone.

Differences in degradation rate constants in different mixing regimes were dramatic (Table 2). Lower rate constants were obtained for physically mixed treatments in Exp. A, compared to unmixed and bioturbated treatments. When animal behavior had little effect on particle transport in Exp. A, most of the added fresh algal material persisted at the sediment-water interface under primarily aerobic conditions. Algal lipids in control and bioturbated treatments in Exp. A degraded at similar rates, as indicated by degradation rate constant ratios (Table 2). In contrast, when added algal material was transported deep into sediments by physical mixing, degradation of algal lipids was dominated by anaerobic pathways. It has been demonstrated that lipids degrade at significantly different rates under aerobic and anaerobic conditions, in either sediments or waters (Sun et al., 1997; Harvey and Macko, 1997a). The ratios of degradation rate constants from control and physically mixed treatments ranged from 3–7 for individual lipids, implying that aerobic degradation in controls was faster than anaerobic degradation in physically mixed treatments. It should be noted that the degradation rates of ^{13}C-labeled algal fatty acids might be underestimated somewhat due to a contribution from newly synthesized bacterial fatty acids. This may be especially true for the physically mixed treatments where growth of bacteria is greatly stimulated.

Table 2
Lipid degradation rate constants (day^{-1}) and correlation coefficient values (r^2) derived from ^{13}C-algae fatty acid and phytol inventories in sediment cores with various treatments during experiment A (three series with five replicates: unmixed control cores; bioturbated cores with three macrofauna species; and cores physically mixed at initial $t = 0$)

Incubations	16:1	16:0	18:1	Phytol
A: unmixed	0.048 (0.67)	0.062 (0.81)	0.069 (0.83)	0.051 (0.89)
A: animal mixed	0.039 (0.75)	0.046 (0.85)	0.055 (0.93)	0.048 (0.96)
A: physical mixed	0.007 (0.57)	0.009 (0.82)	0.023 (0.94)	0.014 (0.48)
Unmixed/animal	1.23	1.35	1.25	1.06
Unmixed/physical	6.86	6.89	3.00	3.64

To further clarify the difference between aerobic and anaerobic degradation of algal lipids, we estimated degradation rate constants for individual lipids in three different intervals for physically mixed cores from Exp. A. When the sediments were initially mixed, dissolved oxygen and organic tracers were distributed throughout the mixed portion of the core. During incubation, the aerobic/anaerobic boundary moved upwards but particulate organic tracers remained at the depth where they were initially deposited. We divided the sediment profile into three zones representing different redox conditions: 0–0.5 cm (oxic), 0.5–2 cm (suboxic), and 2–7 cm (anoxic). Field observations of redox conditions in sediments near Sapelo Island showed a similar zonation (Lowe et al., 2000; Meile et al., 2001). When the sediments collected from the Sapelo Island area were homogenized and incubated in a similar way to our experiment, redox species profiles also showed distinct redox zonation (Cai et al., 2001). Degradation rate constants calculated for various labeled algal lipids in these zones (Fig. 6) showed clearly that lipids degraded much faster in surface aerobic sediments than in deep anaerobic sediments. A similar depth-dependent degradation pattern for chlorophyll-a was observed by Ingalls et al. (2000).

The extent of degradation for each individual lipid was also different in various mixing regimes. For example, with repeated mixing in Exp. C, almost 50% of saturated 16:0 fatty acid was lost but only small amounts of 16:1 and 18:1 unsaturated fatty acids were degraded during the experiment (Table 1). However, degradation of phytol seemed to vary inversely with the frequency of mixing (Table 1). More frequent mixing led to less loss of phytol. We know that bacterial abundance increased with frequent mixing in this experiment, especially in the surface 0–0.5 cm sediment (Hollibaugh,

unpublished data). But, it is not clear how individual lipids are degraded by mixed microbial assemblages in mixed sediments. On the other hand, essentially all unsaturated 16:1 fatty acid was removed in Exp. B treatments with crustaceans, but degradation of other lipids was the same in all treatments (Table 1). It is known that macrobenthos can enrich or deplete the unsaturated fatty acid content of sediments and that the effect is at least partially species dependent (Bradshaw and Eglinton, 1993). A previous experiment (Harvey et al., 1987) demonstrated that algal unsaturated fatty acids were preferentially assimilated over saturated fatty acid by crustacean grazers. In our Exp. B, high abundances of crustaceans ($\sim$1500/m^2) would result in intensive grazing on the added algal material. Thus, preferential assimilation of unsaturated 16:1 fatty acid by crustaceans is likely responsible for the selective removal of this compound.

4.3. Relevance to microbial metabolism

The production of ^{13}C-labeled bacterial lipids provided insight to microbial metabolic pathways in variable mixing regimes. The first notable feature was the difference in production of ^{13}C-labeled bacterial fatty acids between different coastal environments. It was observed that only one bacterial ^{13}C-labeled fatty acid (*iso*-15:0) was produced in Long Island Sound (LIS) sediments in incubations with similar labeled algae (Sun et al., 1999). However, many ^{13}C-labeled bacterial fatty acids, including *iso*-, *anteiso*-15:0, and 15:0 and 17:0 were detected in the experiments reported here. Moodley et al. (2000) observed that bacteria assimilated 2–4% of the added ^{13}C-labeled algal carbon and produced several ^{13}C-labeled fatty acids (e.g., *iso*-14;0, *iso*-15:0, *anteiso*-15:0, *iso*-16:0, and 20:5) during incubation experiments conducted with Oosterschelde Estuary (Netherlands) sediments. Boschker et al. (1998) demonstrated linkage between production of bacterial fatty acids and microorganisms actively involved in biodegradation using an isotope labeling approach. It can be expected that differences in the types of bacterial fatty acids produced in different environments may be largely dependent on bacterial community structure. However, there has been no direct comparison of the bacterial communities between LIS and Georgia coastal sediments.

The second interesting feature is the effect of mixing regimes on production of ^{13}C-labeled bacterial lipids. For example, in Exp. A, production of *iso*- and *anteiso*-15:0 fatty acids was apparently higher in sediments that were stirred once than in unmixed or bioturbated treatments (Fig. 4). Elevated production of bacterial fatty acids was consistent with greater abundance of bacteria in stirred sediments (Hollibaugh, unpublished data). When the sediments were stirred frequently in Exp. C, the concentration of bacterial fatty acids (Table 1) was somewhat lower, although the bacterial abundance increased with frequency of mixing. The relation between production of ^{13}C-labeled bacterial fatty acids and bacterial abundance in stirred sediments was complicated, and may be regulated by supply and availability of ^{13}C-labeled substrate, redox dynamics, and the turnover rate of bacteria cells. Harvey and Macko (1997b) indicated that total fatty acids attributed to bacteria had no strong relationship with bacterial biomass calculated from measured bacterial

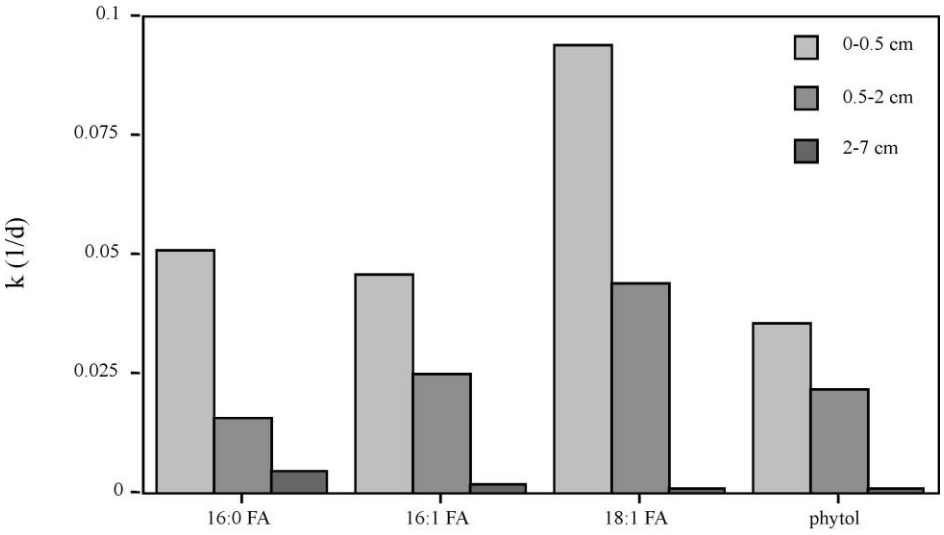

Fig. 6. Comparison of degradation rate constants of various algal lipids estimated from different depth inventories (0–0.5 cm, 0.5–2 cm, and 2–7 cm) in experiment A physically-mixed microcosms.

abundance. On the other hand, the production of bacterial fatty acids seemed to be stimulated by bioturbation. For example, ^{13}C-labeled bacterial fatty acid production in Exp. B was higher in the crustacean treatments than in unmixed or polychaete treatments. It is well known that animal grazing can stimulate rapid growth of microbial populations (Plante et al., 1990). Thus, elevated production of ^{13}C-labeled bacterial fatty acids in the crustacean treatments probably resulted from intensive grazing by this animal.

The third unique feature of these experiments was the production of another ^{13}C-labeled neutral lipid (C_{16} alcohol) in various incubations. Unlike bacterial fatty acids, which were not uniformly labeled, the newly-produced ^{13}C-labeled alcohol was uniformly labeled with ^{13}C. Thus, it was characterized as a transformation product from the ^{13}C-labeled substrate. This compound was initially produced in all treatments during Exp. A and was more rapidly degraded in unmixed and bioturbated treatments than in physically stirred treatments. Under strong mixing regimes, either by repeated stirring or by bioturbation in Exps. B and C, this ^{13}C-labeled alcohol did not accumulate. Small amounts of this ^{13}C-labeled alcohol were observed in unmixed and polycheate treatments. Our previous study (Sun et al., 1999) suggested that the formation of this compound was mediated by microbial processes. However, it is not clear how different mixing processes control the stability of this transformation product.

4.4. Geochemical implications

Our experiments demonstrate that the rate and extent of algal lipid degradation in surface sediments are strongly affected by mixing processes. The key is that different mixing processes result in various geochemical regimes for algal lipid degradation. These geochemical regimes are characterized by different redox conditions. When bloom-produced organic matter reaches the sediment-water interface, mixing processes redistribute labile organic materials into sediment zones with different redox conditions. If some organic compounds such as lipids are redox sensitive, their degradation and preservation would be more or less dependent on mixing, which results in relocation to different redox zones in sediments. Without mixing, the algal organic matter deposited from an oxygenated water column will remain at the aerobic interface and degrade primarily via aerobic pathways, as shown by our control microcosms. Biological mixing (bioturbation) creates a variable redox regime for algal organic matter degradation. Thus, algal lipids degrade via alternative aerobic and anaerobic pathways in the biological mixing zone. Anaerobic pathways include NO_3^- reduction, Mn and Fe reduction, and SO_4^{2-} reduction, depending on availability of these different electron acceptors at different depths. When physical mixing becomes strong enough, algal organic matter on the interface will be rapidly mixed down into anoxic sediments and degrade mainly via anaerobic pathways.

Although our conclusions were derived from laboratory experiments, they could be useful for explaining the difference in degradation rates of lipids observed in various depositional environments. For example, it was observed that planktonic lipids were degraded more rapidly at an oxic and well-bioturbated site than at a hypoxic site in Long Island Sound (Sun and Wakeham, 1999). Biological mixing results in diagenetic properties more characteristic of completely oxidized conditions, in spite of the fact that dissolved oxygen penetrates into the sediment only a few mm (Aller, 1994b). In Cape Lookout Bight (North Carolina, USA), sediment accumulates at a rate of 10 cm/y and organic matter including planktonic lipids is rapidly buried in deep anaerobic sediments (Canuel et al., 1990). Degradation of lipids in deep anaerobic sediments was markedly slower than at the sediment surface, which is exposed to oxygenated waters (Canuel and Martens, 1996). Although rapid sedimentation is mechanistically different from physical stirring, the consequences for lipid degradation might be similar. In the Georgia coastal area, physical mixing can sometimes be very important for benthic processes. It was observed by Hopkinson (1985) that surface sediments off Sapelo Island were mixed down to 25 cm depth when winds exceeded 25 knots. We have observed that vertical profiles of lipids in our sampling site (unpublished data) had distinct characteristics (e.g., sublayer maximum and deeper penetration) compared to bioturbation-dominated sediments. But we cannot distinguish physical mixing from bioturbation because both are significantly important at our study site.

It was proposed by Hartnett et al. (1998) that organic carbon preservation in continental margin sediments is largely controlled by oxygen exposure time in surface sediments. The oxygen exposure time was defined as the depth of oxygen penetration divided by the sedimentation rate. Longer oxygen exposure times result in lower organic carbon preservation. Our results may support this theory at a molecular level. The critical step for algal lipid degradation in surface sediments is how long these compounds persist in the aerobic zone. High sedimentation and episodic stirring enforce the rapid passage of organic compounds through the surficial aerobic layer into the underlying anaerobic sediments, resulting in slow degradation, or high preservation in anaerobic sediments. On the other hand, bioturbation creates a continuous cycling between oxic and anoxic zones, prolonging the exposure time of organic compounds in the aerobic zone and causing rapid degradation of oxygen-sensitive organic compounds. Therefore, the effects of different mixing mechanisms should be considered separately when both biological and physical processes are significantly involved.

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