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Effect of Light on Biomass and Community Structure of Estuarine Detrital Microbiota

R. J. BOBBIE, J. S. NICKELS, G. A. SMITH, S. D. FAZIO, R. H. FINDLAY, W. M. DAVIS, AND D. C. WHITE*

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

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Comparison of estuarine detrital microbiota grown with and without light in the absence of macroscopic grazing showed shifts in the community structure that enabled correlation between various biochemical measures. Analysis of these biochemical measures showed that growth in light induces the smallest increases in procaryotic attributes such as muramic acid; wall glucosamine; lipid phosphate; total extractable adenosine nucleotides; short-branched, cyclopropane, and *cis*-vaccenic fatty acids; lipid glucose and mannose; the incorporation of acetate into lipid; and the formation of deoxyribonucleic acid from thymidine. Measures of the microfauna such as lipid inositol and the γ-linolenic series of polyenoic fatty acids also increased minimally in the light-grown microbiota. Measures of sulfolipid synthesis, lipid glycerol, total extractable palmitate, 18-carbon polyenoic fatty acids, and total polyenoic fatty acids longer than 20 carbons increased 10- to 15-fold in algae and fungi. Chlorophyll *a*, lipid galactose, and the 16- and 20-carbon polyenoic fatty acids characteristic of diatoms increased maximally in the light. This increase of diatom measure correlated with the sheets of diatoms detected by scanning electron microscopy.

Over the past few years it has proved feasible to characterize the marine and estuarine benthic and detrital microbial communities by a biochemical analysis of their biomass, metabolic activities, nutritional status, and community structure. Procaryotic biomass has been estimated by the muramic acid recovered from the hydrolysates of the walls of bacteria and blue-green algae (cyanobacteria) (6, 13, 15, 16). The viable biomass has been estimated by the adenosine triphosphate (ATP) or, better, by the total adenosine nucleotides (4). The membrane biomass has been estimated by the extractable lipid phosphate (26, 27, 29). Metabolic activities can be measured by enzyme activities (26), heterotrophic activities (26, 32), and respiratory activities (26), as well as rate of lipid and deoxyribonucleic acid (DNA) synthesis (24, 28). The nutritional status of the procaryotic microbiota can be estimated in the short term by the energy charge and adenosine-to-ATP ratios (4) or in the long term by the metabolism of poly-β-hydroxybutyrate (9, 20). Community structure can be estimated from the analysis of lipids, particularly from the fatty acids analyzed by capillary gas-liquid chromatography (1, 2, 25). Validation of the assignments of measures of particular components of the microbial community comes from a number of techniques. Isolation of component monocultures from particular environments (with the assumption that the isolation and growth procedures do not change the biochemical composition) have been used (10, 11, 21, 25, 29). Analysis of mixed monocultures of known proportions or manipulation of populations with cultural conditions have been used and shown to correspond with the changes in morphology by scanning electron microscopy (1, 29).

The selective effects of grazers on the microbiota have been analyzed and compared both with the gut and fecal analysis of the predator and with changes in the morphology of the microbiota by scanning electron microscopy (18, 31).

In this study, the detrital microbial assembly was manipulated by controlling the exposure to light. Correlations between the degree of increase in various biochemical measures in the microbiota exposed to light and with the morphology by scanning electron microscopy has given further insight into the community structure of the detrital microbial assembly.

MATERIALS AND METHODS

Materials. Nanograde solvents (Burdick and Jackson, Muskegon, Mich.) and freshly distilled chloroform (Mallinckrodt, St. Louis, Mo.) were used. Reagents were purchased from Pierce Chemical Co. (Rockford,

Ill.), Aldrich Chemical Co. (Milwaukee, Wis.), and Sigma Chemical Co. (St. Louis, Mo.)

Standards. Lipid standards and chromatographic supplies were acquired from Supelco, Inc. (Bellefonte, Pa.) and Applied Science (State College, Pa.). Radioactive reagents were supplied by New England Nuclear Corp. (Boston, Mass.).

Incubation of the samples. Teflon squares (5 cm on each side and 2 mils [51 μm] thick) were strung with nylon fishing line in strings of 30 squares and incubated in Apalachee Bay, Franklin County, Florida (29°5′ N, 84°30′ W) for 8 weeks in the summer of 1979. The strings were then transported to the Florida State University Marine Laboratory in a large vat with oxygenated seawater. Four strands were then placed in each of two 16-liter tanks through which seawater was pumped at a rate of 400 ml/min (95% turnover per day). The seawater conditions during the experiment were salinity, 30‰; dissolved oxygen, 3.2 to 3.4 ppm; pH 7.5 to 7.7; and temperature, 26 to 29°C. One tank was maintained in the dark by a black polyethylene plastic cover and the other received 4,150 lux from a General Electric Grolite lamp on a 14-h-on, 10-h-off photoperiod for 10 days.

Scanning electron microscopy. Teflon squares were removed from the experiments and immediately fixed in glutaraldehyde, critical point dried, coated, and examined with the Cambridge Stereoscan S4-10 microscope (Cambridge Instrument Co., Ossining, N.Y.) as described elsewhere (17). Estuarine water filtered through a 0.45-μm filter (Millipore Corp.) was substituted for the buffering solution.

Lipid extraction. The analytical scheme illustrated in Fig. 1 was used for a portion of the Teflon squares. The cellular lipids were extracted in the dark with a modified Bligh and Dyer method (30); the chloroform was recovered and filtered to remove water. Two samples were removed, with one being analyzed for lipid phosphate and the remaining one stored in the dark for subsequent chlorophyll analysis. The remaining chloroform fraction was dried under nitrogen and hydrolyzed in 1 N HCl for 3 h at 100°C and partitioned against water and chloroform, and the aqueous phase was used for the determination of glycerol and neutral carbohydrates. The organic phase was dried in a stream of nitrogen and subjected to mild acid methanolysis (methanol–concentrated HCl–chloroform, 10:1:1 [vol/vol/vol]) for 1 h at 100°C and partitioned with water and chloroform, and the lipid-containing chloroform phase was dried under nitrogen.

Thin-layer chromatography. After redissolving the lipid in chloroform, the lipids were applied to a 250-μm thin-layer silica gel plate (Whatman K6, 40Å [4 nm]) and fractionated with a solvent of petroleum ether, ethyl ether, and acetic acid (80:20:1, vol/vol/vol). The band containing the fatty acid methyl esters (R_f 5.5 to 6.5) was collected, eluted with chloroform, and dried under nitrogen.

Unsaturated fatty acid methyl esters were separated by argentation thin-layer chromatography. An $AgNO_3$ gradient was prepared on the thin-layer silica gel plates described above by development in $AgNO_3$–ethanol–water (10:70:30, wt/vol/vol) in the dark. The argentated plates were activated at 100°C for 15 min before use. The fatty acid methyl esters were separated into saturated esters (R_f 0.75 to 0.66), monoenoic esters (R_f 0.64 to 0.54), dienoic esters (R_f 0.52 to 0.38), trienoic esters (R_f 0.36 to 0.20), and higher unsaturation ($R_f < 0.20$) by one-dimensional chromatography in heptane–diethyl ether–methanol (90:10:1, vol/vol/vol). Recoveries of authentic 18:1, 18:2, and 18:3 fatty acid methyl esters from the bands of the silver nitrate-impregnated plates averaged 72.7 ± 1.7%.

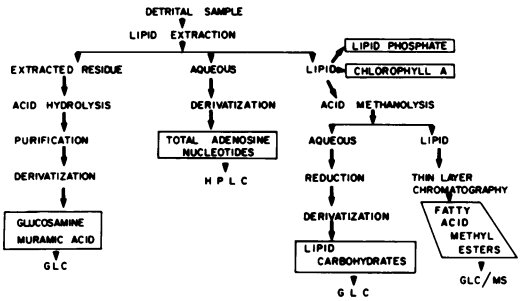

FIG. 1. *Diagram of the analytical scheme for determination of the biomass, activity, and community structure.*

After redissolving in hexane, the fatty acid methyl esters were separated by gas-liquid chromatography with mass spectral analysis.

Gas chromatography. The fatty acid methyl esters to which a methyl nonadecanoate internal standard had been added were separated on a 50-m glass open tubular capillary column coated with Silar 10C (Applied Science Laboratories, State College, Pa.) by using the Varian 3700 gas chromatograph fitted with an autosampler under the conditions described by Bobbie and White (1). A sample of the separation is shown in Fig. 2.

Mass spectrometry. Gas chromatography-mass spectrometry was performed with a Hewlett Packard 5995 instrument with an identical capillary column under similar conditions as described elsewhere (1).

Glycerol and lipid carbohydrates. The glycerol and neutral carbohydrates were derived from the lipids recovered in the aqueous portion of the initial acid hydrolysis (Fig. 1). This aqueous portion was taken to dryness at reduced pressure by using a rotary evaporator (Buchler Instruments, Fort Lee, N.J.). The residual acid was neutralized with 6% (wt/vol) $NaHCO_3$, followed by 0.5 ml of 0.047 M $NaBH_4$ in 0.054 M NaOH, and allowed to stand for 1 h at room temperature. Excess borohydride was destroyed by dropwise addition of 10% (wt/vol) acetic acid, and the sample was added to a 2-g column of Dowex 50 in the H^+ form in a champagne column (Supelco, Inc., Bellefonte, Pa.). Neutral carbohydrates were eluted with 5 ml of water. The remaining borate was removed by adding methanol and evaporating in a stream of nitrogen at 40°C twice. The injection standard, 1,9-nonanediol, was added, and the sugars were peracetylated with acetic anhydride–pyridine (1:1, vol/vol) at 55°C for 60 min. The solvent was carefully removed in a stream of nitrogen; the derivatized carbohydrate was dissolved in chloroform and partitioned twice with 20% (wt/vol) aqueous tartaric acid. The chloroform was recovered and evaporated to dryness under N_2, and the samples

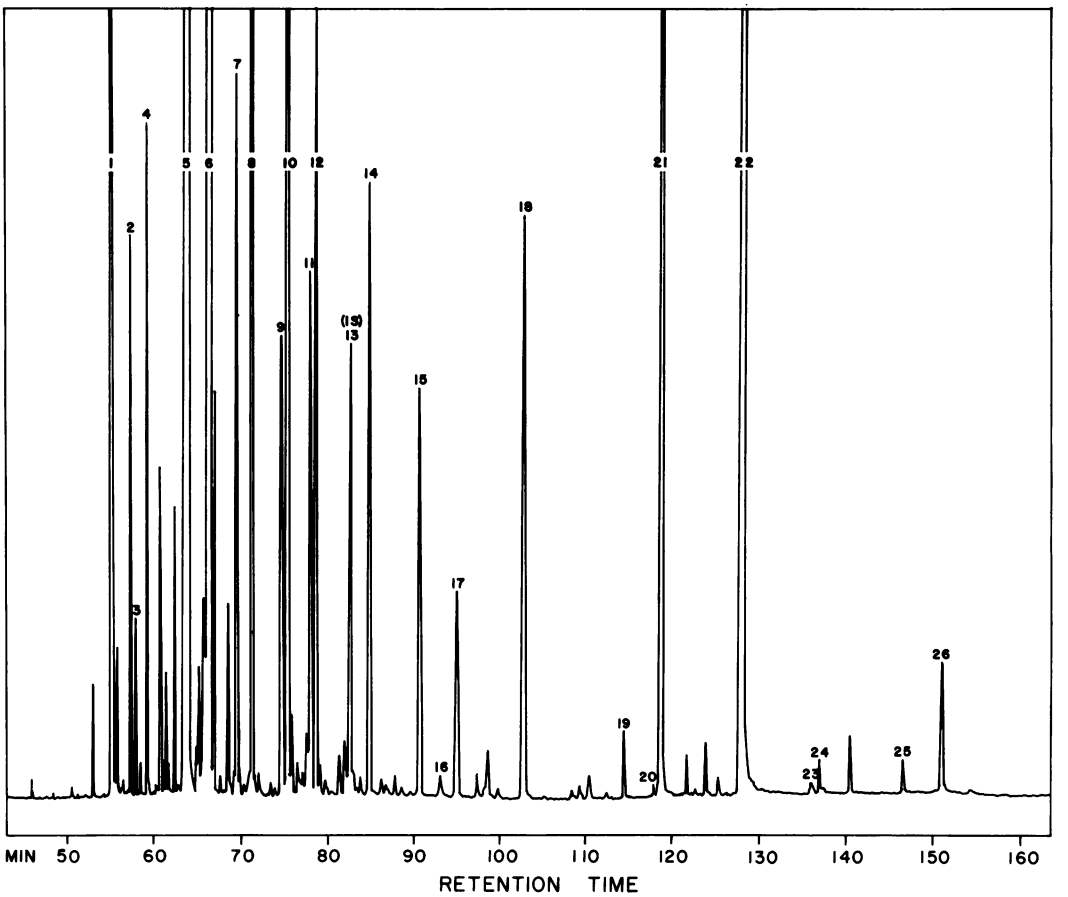

FIG. 2. *Capillary gas chromatographic separation of the fatty acid methyl esters on a 50-m Silar 10C column with the flame ionization detector at a sensitivity of 16 × 10^{-12} A/mV with a splitless injection with 0.5-min venting at 42°C with a temperature increase at 2°C/min to 162°C, isothermal at 162°C for 30 min, followed by a linear increase at 1°C/min to 192°C, which was maintained until all components were eluted. The helium carrier gas flow was 1 ml/min at 16 lb/in^2. Peaks: 1 = 14:0; 2 = i15:0; 3 = a15:0; 4 = 15:0; 5 = 16:0; 6 = 16:1ω7; 7 = 16:2ω7; 8 = 16:2ω4; 9 = 18:0; 10 = 16:3ω4; 11 = 18:1ω9; 12 = 18:1ω7; 13 = 19:0; 14 = 18:2ω6; 15 = 18:3ω6; 16 = 20:0; 17 = 18:3ω3; 18 = 18:4ω3; 19 = 20:3ω6; 20 = 22:0; 21 = 20:4ω6; 22 = 20:5ω3; 23 = 24:0; 24 = 22:4ω6; 25 = 22:5ω3; 26 = 22:6ω3.*

were redissolved in tetrahydrofuran. The derivatized carbohydrates were separated on a 25-m open tubular glass capillary coated with Silar 10C as illustrated in Fig. 3.

Flame ionization detection response factors relative to 1,9-nonanediol and total recovery ($\bar{x}$ ± standard deviation, $n = 5$) for glycerol, rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose, and inositol were 0.5, 74 ± 9.7%; 0.8, 94 ± 8.7%; 0.98, 70 ± 7.2%; 0.83, 73 ± 16.6%; 0.83, 80 ± 11.6%; 0.72, 75 ± 5.9%; 0.83, 88 ± 13%; 0.85, 77 ± 11%; 0.77, 91 ± 16.4%; 0.8, 110 ± 5.5%.

Lipid phosphate. A sample of the lipid from the chloroform fraction of the Bligh-Dyer was digested in 23% (vol/vol) perchloric acid for 2 h at 200°C, and the phosphate was determined colorimetrically (30).

Chlorophyll *a*. A separate sample of the lipid carefully protected from light was dried in a stream of nitrogen and dissolved in acetone–water (9:1, vol/vol) containing magnesium carbonate, and the absorbance between 400 and 800 nm was determined with a Gilford 2400-S recording spectrophotometer in 1-cm path length cuvettes. Chlorophyll *a* concentration was calculated by using a millimolar extinction coefficient of 13.4/cm at 660 nm.

Muramic acid and glucosamine. The Teflon squares after lipid extraction (Fig. 1) were refluxed in 6 M HCl for 4.5 h. The bound lipids were extracted after partitioning in chloroform–water (1:1, vol/vol). The remaining aqueous phase was neutralized to pH 6.5 with 0.02 M NaOH. This enabled interfering humic acids to be removed by centrifugation. The resulting supernatant was eluted through a Dowex 50 (H^+) cation ion-exchange minicolumn, and the muramic

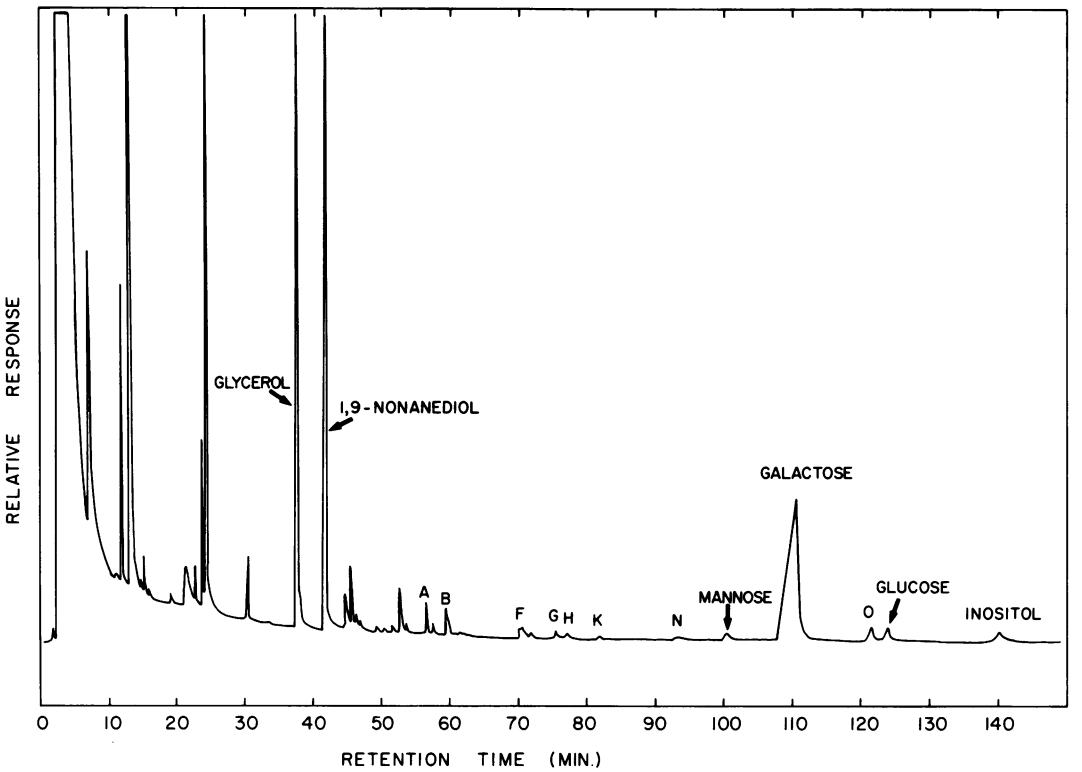

FIG. 3. *Capillary gas chromatographic separation of the peracetylated neutral carbohydrates on a 25-m Silar 10C column with the flame ionization detector at a sensitivity of* 8×10^{-11} *A/mV with a splitless injection with 1-min venting at 45°C, with an increase to 100°C at 5°C/min, then an increase to 190°C at 2°C/min with an isothermal run to the end of the program. The helium carrier gas flow was 1.35 ml/min at 12 lb/in*^{2}.

acid was analyzed as a peracetylated aldonitrile by gas-liquid chromatography (6) as modified by Findlay et al. (R. H. Findlay, R. V. Davis and D. C. White, Abstr. Annu. Meet. Am. Soc. Microbiol., 1981, I21, p. 90).

Adenosine nucleotides. The adenosine nucleotides were recovered quantitatively from the aqueous phase of the Bligh-Dyer extraction (Fig. 1). After lyophilization and subsequent reaction with chloroacetaldehyde, the 1-N^6-ethenoadenosine nucleotides were separated with a phosphate buffer gradient by high-pressure liquid chromatography and assayed by fluorescence as described elsewhere (4).

Radioactive incorporation. At the termination of the incubation, 20 square samples were placed in illuminated or darkened (foil-covered) 2-liter jars containing 400 ml of seawater that had been filtered through 0.22-μm Millipore filters. After the addition of either 400 μCi of [*methyl*-^{3}H]thymidine (120 Ci/mmol), 80 μCi of sodium [1-^{14}C]acetate (45 to 60 mCi/mmol), or 160 μCi of $H_2{}^{35}SO_4$ (43 Ci/mg), the samples were incubated for 4 h. The lipids containing ^{14}C and ^{35}S were extracted, evaporated to dryness, and dissolved in toluene containing 9.28 mM 2,5-bis-(2-(5-tert-butylbenzoxazolyl))-thiophene. The incorporation of [*methyl*-^{3}H]thymidine into DNA was determined after mild alkaline extraction and the purification procedure of Tobin and Anthony (24). Radioactivity was measured with a Packard 2400-S liquid spectrometer (Packard Instrument Co., Downers Grove, Ill.).

RESULTS

Morphology of the detrital microbiota. Incubation of the detrital microbiota with light in the absence of macroscopic grazers stimulated the growth of diatoms (Fig. 4A and B). The dark control showed a sparse community dominated by bacterial microcolonies and thin filaments (Fig. 4C and D).

The morphological differences illustrated in Fig. 4 are reflected in biochemical measures of microbial biomass (Table 1). Neither muramic acid, a component of the cell wall of procaryotic bacteria and blue-green algae, nor glucosamine, a component of both the procaryotic cell wall and the chitin walls of microeucaryotes, showed a significant increase in the detrital microbes exposed to light. Lipid phosphate, however, as a measure of the phospholipids which are confined to membranes (14, 30), was twofold greater in the light.

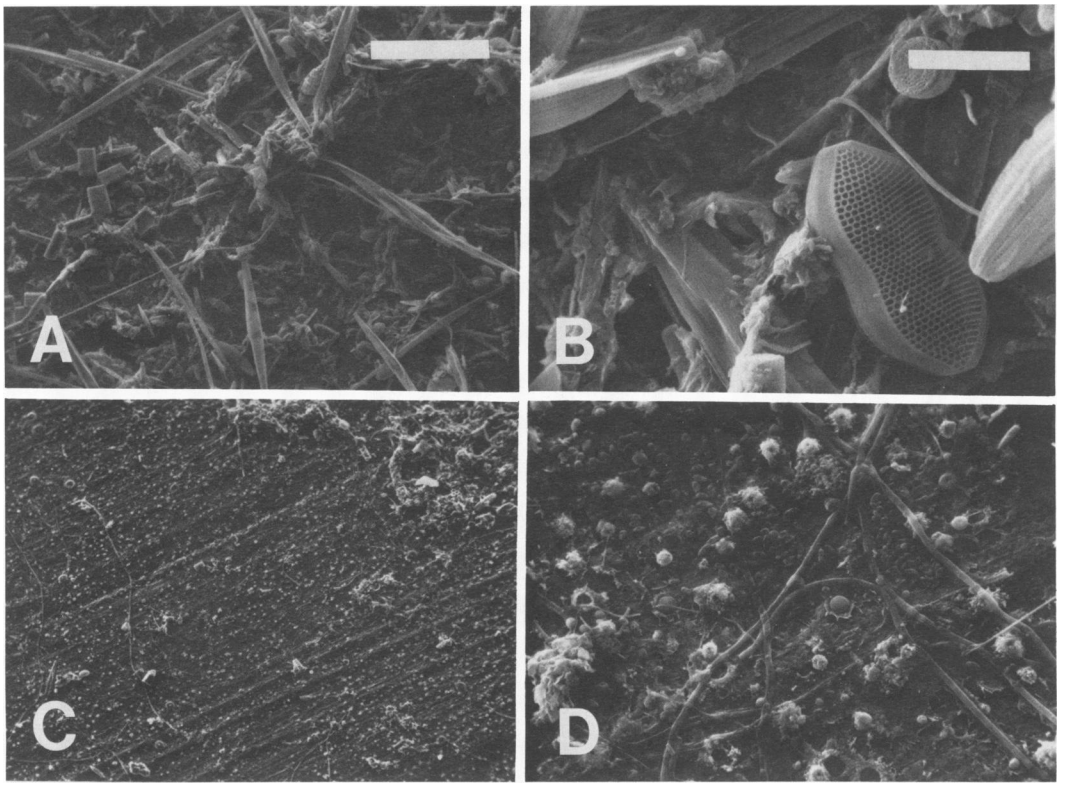

FIG. 4. *Scanning electron micrographs of the estuarine detrital microbiota after 10 days of incubation in the light (A and B) or in the dark (C and D). Magnification of the left-hand column was 153× (A and C) and that of the right-hand column was 1,530× (B and D). Left-hand bar represents 100 μm; right-hand bar represents 10 μm.*

The total extractable adenine nucleotides, a better measure of the microbial biomass than ATP (4), were twofold higher in the light. The lipid glycerol, a rough measure of the eucaryotic endogenous storage material triglyceride, was 15-fold greater, as was the ubiquitous lipid fatty acid palmitic acid. In the estuarine detrital microbiota, the triglyceride glycerol averages between 45 and 100% of the total lipid glycerol (M. J. Gehron and D. C. White, Abstr. Annu. Meet. Am. Soc. Microbiol., 1981, Q134, p. 222). The chlorophyll *a* was 100-fold greater in the light.

Microbial metabolic activities induced by light. The metabolic activities induced by light showed a twofold stimulation of thymidine incorporation into DNA (Table 2). Total lipid synthesis, measured as the incorporation of [^{14}C]acetate into lipids, and the synthesis of sulpholipids were significantly greater in the light. Ratios of total lipid to sulpholipid or DNA synthesis to sulpholipid synthesis were not significantly different.

Effect of light on the community structure of the detrital microbes. The total fatty acids of the microbes, with the exception of the cyclopropane fatty acids typical of stressed bacteria (1), showed striking changes (Table 3). The short iso and anteiso branched saturated fatty acids (a + i 15:0) and the *cis*-vaccenic acid (18:1ω7) that are concentrated in bacteria increased only twofold in the microbiota incubated in the light (Table 3). The polyenoic fatty acids, particularly those associated with diatom monocultures, 16:2ω4, 16:3ω4, and 20:5ω3, increased 30- to 137-fold in the light. The 18-carbon fatty acids which are not found in high concentrations in diatoms (25) increased 2- to 26-fold in the microbiota incubated in the light.

The proportions of some of the fatty acids relative to the total lipid (measured as 16:0) or the phospholipid (measured as lipid phosphate) showed differences between the communities grown in the light and dark (Table 4). The most striking increases in the light-grown communities are in the proportions of 16:2ω4, 16:3ω4, and 20:5ω3 relative to either the total lipid or the

phospholipid. These fatty acids are characteristic of diatoms when diatoms are examined in monoculture. Fatty acids characteristic of bacteria such as a + i 15:0, Δ17:0, Δ19:0, and 18:1ω7, when compared as ratios of the total bacterial community (measured as 15:0), the total lipid from the whole community (measured as 16:0), the portion of the bacterial and eucaryotic community that contains oleic acid (measured as 18:1ω9), or the total phospholipid (measured as the lipid phosphate), showed very little change between light and dark (0.1- to 1.5-fold change). The proportions of the sum of all the 18-carbon fatty acids, 20:4ω6, 22:5ω3, and 22:6ω3, showed a slightly larger increase in the light than did the bacterial fatty acids. The proportions of the rest of the fatty acids showed a slightly greater increase in the light-grown community than either these or the bacterial fatty acids.

Lipid neutral carbohydrates. The lipid-derived carbohydrates showed significant changes induced by incubation in the light (Table 5). All of the components with the exception of A, F, O, glucose, and inositol were enriched in the lipids from the light-incubated microbiota. Particularly striking was the increase in carbohydrate H and galactose.

***trans*-Fatty acids.** To refine the analysis of the fatty acids derived from the microbiota a separation by silver nitrate-impregnated thin-layer plates before gas-liquid chromatographic analysis was performed. The monoenoic fatty ester band was recovered and examined for *trans*-monoenoic fatty acids which have recently been found in some marine bacteria (21). With authentic 16:1ω7 *cis* and *trans* and 18:1ω9 *cis* and *trans* as markers with an isothermal analysis, no significant *trans*-fatty acids were detected in the lipids of detritus from light- or dark-exposed microbial communities. The 16:1ω13 (*trans*) from the phosphatidylglycerol of the photosystem II (8) apparently overlapped another component, since it was not detectable by gas chromatography-mass spectroscopy.

TABLE 1. *Effect of light on the detrital microbial biomass*

Measure	Microbial biomass (nmol/square)		Ratio (light/dark)
	Light	Dark	
Lipid phosphate	63 (13)[a]	27 (5)***[b]	2.3
Muramic acid	3.7 (1.4)	2.3 (1.4)	1.6
Wall glucosamine	32 (11)	21 (7)	1.5
Total adenylate	0.93 (0.17)	0.38 (0.11)***	2.4
Lipid glycerol	12 (4.6)	0.8 (0.3)***	15.1
Lipid palmitate	106 (21)	6.8 (0.5)***	15.7
Chlorophyll *a*	2.2 (0.22)	0.024 (0.006)***	92.5

[a] $\bar{x}$ (standard deviation), four samples per entry, on a 25-cm² Teflon square incubated for 2 weeks.

[b] *, **, and *** indicate statistically significant differences between the means at the 0.1, 0.05, and 0.01 levels by analysis of variance.

TABLE 2. *Effect of light on detrital metabolic activity*

Measure	Microbial metabolic activity (cpm/square per 4 h)		Ratio (light/dark)
	Light	Dark	
^{3}H into DNA	2,160 (392)[a]	1,100 (227)***[b]	1.9
^{14}C into lipid[c]	509 (70)	207 (33)***	2.5
^{35}S into lipid	714 (670)	134 (98)**	5.3

[a] See footnote *a*, Table 1.

[b] See footnote *b*, Table 1.

[c] Counts per minute × 10^3.

TABLE 3. *Effect of light on the fatty acid composition of the estuarine detrital microbiota*

Fatty acid	Amt (nmol/square)		Ratio (light/dark)
	Light	Dark	
a + i 15:0[a]	3.83 (0.86)[b]	1.1 (0.21)***[c]	3.5
Δ(17:0 + 19:0)	0.18 (0.07)	0.13 (0.05)	1.4
18:1ω7	9.45 (1.82)	4.61 (0.72)***	2.0
16:1ω7	111.0 (32.7)	3.45 (0.51)***	32.2
16:2ω7	5.34 (2.3)	0.19 (0.03)***	28.1
16:2ω4	13.9 (4.4)	0.39 (0.04)***	35.6
16:3ω4	31.4 (13)	0.23 (0.08)***	137.0
18:2ω6	7.28 (3.63)	0.4 (0.05)***	18.2
18:0,1,2,3,4[d]	35.8 (13.3)	7.02 (0.74)***	5.1
18:3ω3	2.33 (1.37)	0.09 (0.07)**	26.0
18:4ω3	5.73 (3.32)	0.22 (0.06)**	26.0
20:5ω3	47.4 (24.1)	1.05 (0.22)***	45.0
20:4ω6	18.0 (9.2)	1.16 (0.26)**	16.0
22:5ω3	0.39 (0.19)	0.10 (0.03)**	4.0
22:6ω3	1.68 (0.95)	0.37 (0.11)**	4.5
Total polyenoics > 20[e]	68.0 (33)	2.98 (0.63)***	22.8
Polyenoic ω6/ω3	0.43 (0.06)	0.87 (0.06)***	0.57

[a] Fatty acids are designated as a or i for anteiso or iso branched, Δ for cyclopropane; number of carbon atoms: number of double bonds, position of the unsaturation closest to the ω end, separated as illustrated in Fig. 2.

[b] $\bar{x}$ (standard deviation), four samples per entry.

[c] Significance as in Table 1.

[d] Total octadecanoic fatty acids with 0, 1, 2, 3, and 4 double bonds.

[e] Total polyenoic fatty acids = the sum of 20:3ω6, 20:5ω3, 20:4ω6, 22:4ω6, 22:5ω3, and 22:6ω3.

DISCUSSION

Relationships between measures of microbial biomass. The effect of light on the detrital microbiota includes increases in the microbial biomass markers of Tables 1 and 2 to three distinct degrees. Light increases lipid phosphate, muramic acid, wall glucosamine, and total extractable adenylate 1- to 3-fold, lipid glycerol and total palmitate 15-fold, and chlorophyll *a* 92-fold. This distinction between the biomass measures is even more readily detectable if the

TABLE 4. *Effect of light on the community structure of the estuarine detrital microbiota measured as ratios of fatty acids to total palmitate and lipid phosphate*

Fatty acid ratios	Light	Dark	Ratio, fatty acid to 16:0, light/dark[a]	Ratio, fatty acid to lipid phosphate, light/dark[b]
a + i 15:0/15:0[c]	1.09 (0.08)[d]	1.88 (0.31)***[e]	0.6	
a + i 15:0/16:0	0.04 (0.009)	0.16 (0.03)***	0.25	1.49
Δ(17:0 + 19:0)/16:0	0.002 (0.002)	0.02 (0.007)***	0.1	0.58
18:1ω7/16:0	0.09 (0.006)	0.69 (0.13)***	0.13	0.88
18:1ω7/18:1ω9	1.54 (0.11)	2.99 (0.31)***	0.52	
16:1ω7/16:0	1.05 (0.14)	0.51 (0.07)***	2.1	12.8
16:2ω7/16:0	0.05 (0.01)	0.03 (0.01)***	1.7	12.2
16:2ω4/16:0	0.13 (0.02)	0.06 (0.01)***	2.2	15.7
16:3ω4/16:0	0.29 (0.08)	0.03 (0.01)***	9.0	47.1
18:2ω6,/16:0	0.07 (0.02)	0.06 (0.007)	1.2	7.8
18:0,1,2,3,4/16:0[f]	0.37 (0.06)	1.32 (0.15)***	0.28	2.19
18:3ω3/16:0	0.02 (0.01)	0.01 (0.01)*	1.5	11.2
18:4ω3/16:0	0.05 (0.02)	0.03 (0.01)**	1.7	11.2
20:5ω3/16:0	0.43 (0.17)	0.16 (0.04)***	2.7	18.9
20:4ω6/16:0	0.16 (0.07)	0.17 (0.05)	0.9	6.7
22:5ω3/16:0	0.003 (0.002)	0.015 (0.006)***	0.2	1.6
22:6ω3/16:0	0.02 (0.01)	0.06 (0.02)***	0.3	1.9
Total polyenoics > 20[g]/16:0	0.62 (0.24)	0.41 (0.11)*	1.5	9.8

[a] Ratio of the ratio of nanomoles of fatty acid per square to the total 16:0 in the light (106 nmol/square) versus the ratio of nanomoles of fatty acid per square to the total 16:0 in the dark (6.8 nmol/square).

[b] Ratio of the ratio of nanomoles of fatty acid per square to the extractable lipid phosphate in the light (63 nmol/square) versus the ratio of nanomoles of fatty acid per square of the total extractable lipid phosphate in the dark (27 nmol/square).

[c] Fatty acids are designated as a or i for anteiso or iso branched and Δ for cyclopropane; number of carbon atoms: number of double bonds, position of the unsaturation closest to the ω end separated as illustrated in Fig. 2.

[d] $\bar{x}$ (standard deviation), four samples per entry.

[e] Significance as in Table 1.

[f] Ratio of total octadecanoic fatty acids with 0, 1, 2, 3, and 4 double bonds.

[g] Total polyenoic fatty acids = the sum of 20:3ω6, 20:5ω3, 20:4ω6, 22:4ω6, 22:5ω3, and 22:6ω3.

TABLE 5. *Effect of light on the carbohydrates recovered after acid hydrolysis of the lipids extracted from the detrital microbiota*

Component	Amt (nmol/square)		Ratio, light/dark
	Light	Dark	
A[a]	0.19 (0.11)[b]	0.07 (0.03)	2.7
B	0.15 (0.13)	0.00 (0.0)	
E	0.74 (0.51)	0.00 (0.0)	
F	0.11 (0.007)	0.21 (0.15)	0.5
G	0.95 (0.68)	0.00 (0.0)	
H	2.23 (0.28)	0.04 (0.00)**[c]	55.0
K	0.53 (0.46)	0.00 (0.0)	
N	0.39 (0.62)	0.24 (0.00)**	1.6
Mannose	0.43 (0.44)	0.11 (0.02)*	3.9
Galactose	13.5 (6.1)	0.00 (0.0)***	
O	0.05 (0.03)	0.05 (0.001)	1.0
Glucose	0.57 (0.26)	0.82 (0.38)	0.69
Inositol	0.1 (0.04)	0.1 (0.04)	1.0

[a] Letters refer to the components with elution volumes indicated in Fig. 3.

[b] $\bar{x}$ (standard deviation), four points per entry.

[c] Statistical significance as in Table 1.

effects of light on the ratios of biomass measures are used. This is illustrated by the data in Table 6.

Relationships between microbial biomass and community structure. The fatty acids derived from lipid extractions of the detrital microbiota can be used to indicate changes in microbial community structure (1). Examination of the effect of light on the changes in the concentrations of fatty acids is given in Table 3. Again three distinct degrees of increases are detected: 1- to 5-fold for a + i 15:0, Δ17:0 + Δ19:0, 18:1ω7, 22:5ω3, 22:6ω3 and the 18-carbon fatty acids; 137-fold for 16:3ω4; and 16- to 35-fold for the rest. Comparing the effect of light on the increases in the ratios of fatty acids to 16:0 and to lipid phosphate shows the three categories of changes induced by light more clearly (Table 4).

With the analyses of the effects of light on the microbial community structure, three general communities with different responses to light can be suggested (Table 7).

(i) Smallest increases. The procaryotes measured by the unique cell wall component muramic acid (6, 13) increase least. Corresponding to this increase is the lipid phosphate and the total adenosine nucleotides. Both the phospholipid and in most cases total adenosine nucleotides per gram (dry weight) are highest in bacteria among microbes (4, 26, 30). Glucosamine is part of the muramyl peptide and is also found in microeucaryotic chitin walls. The chitin walls do not hydrolyze under the conditions of this analysis. Short-branched cyclopropane and *cis*-vaccenic acids are characteristic particularly of the various components of the procaryotes (1, 21). Thymidine incorporation into DNA amongst the microbes is a procaryotic activity, as macroeucaryotes lack the salvage pathway (24, 29). Lipid carbohydrates rich in glucose and mannose are enriched in bacteria (23).

Polyenoic fatty acids and phosphatidylinositol are characteristic of microeucaryotes (5, 12). The microfauna, such as nematodes, from this area are enriched in 18:0, 20:4ω6 and 22:5ω3 (R. J. Bobbie, Ph.D. thesis, Florida State University, Tallahassee, 1980).

(ii) Intermediate increase. At least half the total lipid glycerol as measured after mild acid methanolysis represents the triglyceride. Glycerol phosphate is not hydrolyzed under these conditions (Gehron and White, Abstr. Annu. Meet. Am. Soc. Microbiol., 1981, Q134, p. 222). Palmitate is a ubiquitous fatty acid found in large amounts in almost all microbes (12). It is a measure of the total lipid which includes the neutral lipids. Neutral lipids are generally not found to any great extent in the eubacteria. Linoleic acid (18:2ω6) is found in fungi, flexibacteria, and algae (5, 12). Eucaryotic algae contain 18:3ω3, 18:4ω3 and a high level of sulpholipid in the photosynthetic apparatus (7, 19). Microcosms in which microeucaryotes were stimulated were enriched in polyenoic fatty acids longer than 20 carbons and in the rate of ^{35}S incorporated into sulpholipids (29), suggesting these measures correlate with microeucaryote activities.

(iii) Largest increase. Largest increases in the biochemical measures induced by light are characteristic of the diatoms, which are clearly visible in the scanning electron micrographs (Fig. 4). Diatoms contain chlorophyll *a* and lipid galactose in the monogalactosyl diglyceride of photosystem II (22) as reflected in the analysis. Diatoms do not contain significant amounts of 18-carbon fatty acids but are particularly enriched in 16-carbon polyenoics, especially 16:3ω4, and 20:5ω3 fatty acids (3, 25).

TABLE 6. *Comparison of ratios of biomass measures of the detrital microbiota between light and dark*

Component	Light/dark ratio of component to:			
	Palmitate	Lipid phosphate	Lipid glycerol	Chlorophyll *a*
Lipid phosphate	0.15		0.26	0.025
Muramic acid	0.07	0.67	0.11	0.017
Wall glucosamine	0.10	0.65	0.11	0.017
Total adenylate	0.15	1.05	0.17	0.026
Lipid glycerol	0.92	6.12		0.15
Lipid palmitate		6.72	1.07	0.17
Chlorophyll *a*	6.0	38.6	6.31	

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TABLE 7. *Relative increases in biomass and community structure measures*

Biomass increase	Community	Component measured
Small	Bacteria-procaryote	Lipid phosphate, muramic acid, wall glucosamine, total adenylate
		a + i 15:0, Δ17:0, Δ19:0, 18:1ω7
		Lipid carbohydrates A, N, mannose, O, glucose
		[^{3}H]thymidine into DNA, [^{14}C]acetate into lipid
	Microfauna	Lipid inositol
		18:0, 18:1, 20:4ω6, 22:5ω3, 22:6ω3
Intermediate	Microfloral-fungal	Lipid glycerol, total palmitate
		16:1ω7, 16:2ω7, 16:3ω4, 18:2ω6, 18:3ω3, 18:4ω3
		Total polyenoics > 20 carbon atoms
		^{35}S into lipid
Large	Diatom	Chlorophyll *a*
		16:3ω4, 20:5ω3
		Lipid carbohydrates E, H, galactose

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LITERATURE CITED

1. **Bobbie, R. J., and D. C. White.** 1980. Characterization of benthic microbial community structure by high-resolution gas chromatography. Appl. Environ. Microbiol. **39**:1212–1222.
2. **Boon, J. J., J. W. de Leeuw, G. T. v. d. Hoek, and J. H. Vosjan.** 1977. Significance and taxonomic value of iso and anteiso monoenoic fatty acids and branched β-hydroxy fatty acids in *Desulfovibrio desulfuricans*. J. Bacteriol. **129**:1183–1191.
3. **Darley, W. M.** 1977. Biochemical composition, p. 198–223. *In* D. Werner (ed.), The biology of diatoms. University of California Press, Berkeley.
4. **Davis, W. M., and D. C. White.** 1980. Fluorometric determination of adenosine nucleotide derivatives as measures of the microfouling, detrital and sedimentary microbial biomass and physiological status. Appl. Environ. Microbiol. **40**:539–548.
5. **Erwin, J. A.** 1973. Fatty acids in eukaryotic microorganisms, p. 41–143. *In* J. A. Erwin (ed.), Lipids and biomembranes of eukaryotic microorganisms. Academic Press, Inc., New York.
6. **Fazio, S. D., W. R. Mayberry, and D. C. White.** 1979. Muramic acid assay in sediments. Appl. Environ. Microbiol. **38**:349–350.
7. **Haines, T. H.** 1973. Sulfolipids and halosulfolipids, p. 195–232. *In* J. A. Erwin (ed.), Lipids and biomembranes of eukaryotic microorganisms. Academic Press, Inc., New York.
8. **Harwood, J. L., and A. T. James.** 1975. Metabolism of trans-3-hexadecanoic acid in broad bean. Eur. J. Biochem. **50**:325–334.
9. **Herron, J. S., J. D. King, and D. C. White.** 1978. Recovery of poly-β-hydroxybutyrate from estuarine microflora. Appl. Environ. Microbiol. **35**:251–257.
10. **Johns, R. B., and G. J. Perry.** 1977. Lipids of the marine bacterium *Flexibacter polymorphus*. Arch. Microbiol. **114**:267–271.
11. **Johns, R. B., G. J. Perry, and K. S. Jackson.** 1977. Contribution of bacterial lipids to recent marine sediments. Estuarine Coastal Mar. Sci. **5**:521–529.
12. **Kates, M.** 1964. Bacterial lipids. Adv. Lipid Res. **2**:17–90.
13. **King, J. D., and D. C. White.** 1977. Muramic acid as a measure of microbial biomass in estuarine and marine samples. Appl. Environ. Microbiol. **33**:777–783.
14. **King, J. D., D. C. White, and C. W. Taylor.** 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Appl. Environ. Microbiol. **33**:1177–1183.
15. **Moriarty, D. J. W.** 1975. A method for estimating the biomass of bacteria in aquatic sediments and its application to trophic studies. Oecologia **20**:219–229.
16. **Moriarty, D. J. W.** 1977. Improved method using muramic acid to estimate biomass of bacteria in sediments. Oecologia **26**:317–323.
17. **Morrison, S. J., J. D. King, R. J. Bobbie, R. E. Bechtold, and D. C. White.** 1977. Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, USA. Mar. Biol. **41**:229–240.
18. **Morrison, S. J., and D. C. White.** 1980. Effects of grazing by estuarine gammaridean amphipods on the microbiota of allochthonous detritus. Appl. Environ. Microbiol. **40**:659–671.
19. **Nichols, B. W.** 1970. Comparative lipid biochemistry of photosynthetic organsims, p. 105–118. *In* J. B. Harborne (ed.), Phytochemical phylogeny. Academic Press, Inc., New York.
20. **Nickels, J. S., J. D. King, and D. C. White.** 1979. Poly-β-hydroxybutyrate metabolism as a measure of unbalanced growth of the estuarine detrital microbiota. Appl. Environ. Microbiol. **37**:459–465.
21. **Perry, G. J., J. K. Volkman, and R. B. Johns.** 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Acta **43**:1715–1725.
22. **Rosenberg, A.** 1973. Chloroplast lipids of photosynthesizing eukaryotic protists, p. 235–258. *In* J. A. Erwin (ed.), Lipids and biomembranes of eukaryotic organisms. Academic Press, Inc., New York.
23. **Shaw, N.** 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol. **17**:63–108.
24. **Tobin, R. S., and D. H. J. Anthony.** 1978. Tritiated thymidine incorporation as a measure of microbial activity in lake sediments. Limnol. Oceanogr. **23**:161–165.
25. **Volkman, J. K., and R. B. Johns.** 1977. The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment. Nature (London) **267**:693–694.
26. **White, D. C., R. J. Bobbie, J. S. Herron, J. D. King, and S. J. Morrison.** 1979. Biochemical measurements of microbial mass and activity from environmental samples, p. 69–81. *In* J. W. Costerton and R. R. Colwell (ed.), Native aquatic bacteria: enumeration, activity and ecology. ASTM STP 695. American Society for Testing and Materials, Philadelphia.
27. **White, D. C., R. J. Bobbie, J. D. King, J. Nickels, and P. Amoe.** 1979. Lipid analysis of sediments for microbial biomass and community structure, p. 87–103. *In* C. D. Litchfield and P. L. Seyfried (ed.), Methodology for biomass determinations and microbial activities in sediments. ASTM STP 673. American Society for Testing and Materials, Philadelphia.
28. **White, D. C., R. J. Bobbie, S. J. Morrison, D. K. Oosterhof, C. W. Taylor, and D. A. Meeter.** 1977. Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. Limnol. Oceanogr. **22**:1089–1099.
29. **White, D. C., R. J. Bobbie, J. S. Nickels, S. D. Fazio, and W. M. Davis.** 1980. Non-selective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. Bot. Mar. **23**:239–250.
30. **White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie.** 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia **40**:51–62.
31. **White, D. C., R. H. Findlay, S. D. Fazio, R. J. Bobbie, J. S. Nickels, W. M. Davis, G. A. Smith, and R. F. Martz.** 1980. Effects of bioturbation and predation by *Mellita quinquiesperforata* on the sedimentary microbial community structure, p. 163–171. *In* V. S. Kennedy (ed.), Estuarine perspectives. Academic Press, Inc., New York.
32. **Wright, R. T., and J. E. Hobbie.** 1965. The uptake of organic solutes in lake water. Limnol. Oceanogr. **10**:22–28.