# Laboratory study of disturbance in marine sediments: response of a microbial community

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ABSTRACT Disturbance has been shown to be an important component of the ecology of soft-bottom macrobenthic and meiobenthic marine communities. Its importance in the ecology of microbial communities was investigated by using sieving of marine sediments as a controlled disturbance. Following the disturbance, sediments were maintained in microcosms. Using a suite of biochemical measures, sieving was found to influence microbial biomass, community structure, and metabolic activity. Sieving caused an immediate decrease in microbial growth rates and a shift in metabolic status towards the synthesis of phospholipid. Microbial biomass was initially unaffected. Several hours later, growth rates increased and biomass had decreased by 75 %. Microbial biomass returned to pre-disturbance levels 8 h after sieving. Groups of phospholipid, ester-linked fatty acids, each associated with different functional groups of microorganisms, varied in their response to sieving. This result suggested that components of the microbial community differed in their reaction to this disturbance. Ambient sediments collected at the time of the construction of the microcosms were contrasted with sediments maintained in the laboratory microcosms for 5 d. Laboratory conditions significantly altered the microbial community structure and growth rates were significantly lower. Measures of metabolic status indicated that some of the microorganisms were stressed. This study demonstrates the potential significance of disturbance in the ecology of the benthic microbial community and that uncoupling sediment from the biotic and abiotic influences of the environment significantly affects the composition and activity of the microbial community.

## INTRODUCTION

Sedimentary bacteria live in a physically and chemically complex environment and numerous factors have been shown to influence their distribution. Microscopic analysis indicates that marine sediments are a heterogeneous environment of organic and inorganic particles of various sizes, bridged, or encompassed, by an organic matrix (Frankel & Mead 1973, Watling 1988). Sediment grain size, the mineral nature of the grains, and the microtopography of the grains have been shown to influence the bacterial biomass in marine sediments (Meadows & Anderson 1967, Dale 1974, Weise & Rheinheimer 1978, Nickels et al. 1981, DeFlaun & Mayer 1983). In shallow waters, deposition

rates of detrital carbon are high and the majority of the decomposition of detritus occurs in the sediments (Suess 1976). The major, early diagenetic reactions result from microbiological decomposition of organic matter (Berner 1976), and this activity produces many complex gradients within the sediments which in turn affect the distribution and metabolic activities of the bacteria there (Jones 1979).

Most, but not all, marine sediments are inhabited by macrobenthos and their activities profoundly affect the physical and chemical nature of the sediments. Burrowing for shelter or food and ingestion of sediment – disturbances caused by biological agents and henceforth referred to as biotic disturbance – cause changes in the grain size, sorting, fabric, water content and compaction of the sediments (for reviews see Gray 1974, Rhoads 1974, Aller 1978, Rhoads & Boyer 1982). Biotic disturbance also affects diagenetic reactions and the porewater profiles of reactants and products (Aller 1982). Sandy sediments may have very high rates of biotic disturbance with turnover rates of 0.7 to 4 d for

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the top 1 cm of sediment (Meyer 1977). While several studies have examined the effects of tubes and burrows of infauna on microbial activity and biomass (e.g. Aller & Yingst 1985, Dobbs & Guckert 1988a), few have directly examined the short-term (hours) effects of biotic disturbance (e.g. Findlay et al. 1985). Findlay et al. (1985) suggested that disturbance of marine sediments would, in a matter of hours, induce significant changes in the microbial community. To demonstrate the potential of disturbance as an important controlling factor in structuring microbial communities in sediments we have employed a laboratory microcosm experimental design within which sieving serves as a controlled disturbance. We choose sieving as a controlled disturbance to simulate the disturbance caused by biotic agents that translocate large amounts of sediments (e.g. feeding rays).

An additional objective of this study was to document the changes induced in the microbial community by construction and maintenance of laboratory microcosms. Sieving of marine sediments is a common practice in construction of sediment microcosms (e.g. Findlay & White 1983a, Aller & Yingst 1985). The use of microcosms has become increasingly important to the study of microbial ecology as it allows a measure of control unattainable in the environment (Pritchard & Bourquin 1984). However, several studies have suggested that important changes can occur in the microbial community of sediments maintained in microcosms (Reichgott & Stevenson 1978, Findlay & White 1983a, Sayler et al. 1983, Federle et al. 1986).

Toward these ends, we used an integrated biochemical approach for the measurement of microbial biomass and rates of growth. The application of these techniques has been reviewed by White (1983, 1986). These techniques are based on measurement of the chemical components of microbial cells. They allow assessment of microbial biomass, community structure, rates of growth, and short- and long-term metabolic status without the problems associated with direct enumeration or culture methods (White 1986). A functional-group analysis, a technique successfully applied in a variety of ecological studies, was used to interpret changes in microbial community structure (e.g. Steneck & Watling 1982, Dobbs & Guckert 1988a). These techniques were used to compare estuarine sediments to those sieved and maintained in laboratory microcosms for up to 5 d.

## MATERIAL AND METHODS

**Materials.** 1-($^{14}$C)-acetate (56 µCi µmol$^{-1}$) was supplied by New England Nuclear, Boston, MA, USA. Unisil brand silicic acid was obtained from Clarkson

Chemical Co., Williamsport, PA, USA. Glass-distilled solvents (Burdick und Jackson, Muskegon, WI, USA) or freshly redistilled analytical grade chloroform (Mallinkrodt, St. Louis, MO, USA) were used. Derivatizing reagents and authentic standards were purchased from Pierce Chemical Co., Rockford, IL, and Aldrich Chemical Co., St. Louis, MO. Fused silica Capillary GLC Durabond columns (DB-1) were supplied by J&W Laboratories, Roncho Cordova, CA, USA.

**Study site.** Sediments used for the sieving disturbance experiment were obtained in July 1983 from an intertidal sandy-bottom site adjacent to the Florida State University Marine Laboratory, Franklin County, Florida, USA (29° 52′ N, 84° 31.5′ W).

**Experimentation.** Two adjacent 1 m$^2$ quadrats were established ca 10 m from shore (mean high water). One quadrat was sampled. Nine cores were taken and 3 replicate cores were randomly assigned to each of 3 assays. Samples were returned to the marine laboratory for processing. These samples served to characterize the ambient sediments. The top 2 cm (approximate) of sediment was removed from the second quadrat and returned to the marine laboratory for sieving. The sediment was passed through a 998 µm sieve using a minimum of seawater. The sieved sediment was thoroughly mixed by hand and placed into 48 numbered 250 ml Kimax beakers. The microcosms were maintained in a laboratory greenhouse in 20 cm of flowing seawater (1000 ml min$^{-1}$).

Beakers were randomly assigned to a sample time and replicate number. Six extra beakers were also scored for replacement, if needed, of any lost beakers. Three replicate beakers were sampled at each sample time. Beakers were sampled immediately after placement in the greenhouse and at 2, 4, 8, 12, 48, 72, and 120 h later. Three 2.5 cm ID cores were taken from each beaker One core was used for lipid analysis, one core for $^{14}$C-acetate incorporation into lipid, and one core for methyl-($^3$H)-thymidine incorporation into DNA. In all cases the top 2 cm of sediment was analyzed. Samples, when necessary, were inoculated and incubated on site. All samples were fixed and transported on ice to the main campus laboratory for processing.

**Lipid extraction.** Sediments were extruded into solvent-rinsed 300 ml polypropylene centrifuge bottles containing 75 ml methanol, 37.5 ml chloroform and 25 ml phosphate buffer (50 µM, pH 7.4) (White et al. 1979). The mixtures were allowed to stand for 24 h at 4 °C as a single phase. The sediments and extraction mixtures were transferred to separatory funnels and the centrifuge bottles washed twice with 18.75 ml of chloroform, followed by 3 washes of 12.5 ml of water All washes were added to the separatory funnels, shaken vigorously, and the extraction mixture allowed to separate into 2 phases overnight. The chloroform layer

(containing the lipid) was filtered through a tared Whatman 2V filter, recovered, and dried. The sediment was recovered in the filter, dried to a constant weight and dry weight determined gravimetrically.

**Lipid partitioning.** The dried lipid was resuspended in 1 ml of chloroform and two 100 μl aliquots removed for analysis of total phospholipid phosphate (LPO4). The remaining lipid was placed on a previously prepared silicic acid column. The preparation of the column and the partitioning of the lipid into neutral lipid, glycolipid and phospholipid fractions were as described in Findlay & White (1987).

**Phospholipid phosphate.** Lipid samples were digested in 23 % (v/v) perchloric acid for 2 h at 200 °C and the phosphate determined colorimetrically (White et al. 1979).

**Ester-linked phospholipid fatty acids.** Ester-linked, phospholipid fatty acids (PLFA) were analyzed as their methyl esters using a modification of the procedures of Bobbie & White (1980). The dried phospholipid fraction was subjected to mild alkaline transesterification and the resulting fatty acid methyl esters purified by thin-layer chromatography. The purified fatty acid methyl esters were identified and quantified using a Varian 3700 gas chromatograph equipped with an autosampler, flame-ionization detector, and a 60 m DB-1 fused-silica capillary column. The abundances of all identified fatty acid methyl esters were summed to yield total ester-linked, phospholipid fatty acids (TPLFA).

**Poly-beta-hydroxyalkanoates** (PHA) were analyzed using the chloroform-methanol extraction method described in Findlay & White (1987).

**Muramic acid.** The sediment was recovered after lipid extraction and analyzed for muramic acid (MA) as described in Findlay et al. (1983).

**Radioactive incorporations.** The ratio of the relative rates of 1-($^{14}$C)-acetate incorporation into PLFA to incorporation into PHA (14CPLFA/14CPHA) was determined using the injection technique described in Findlay et al. (1985). The point of injection was 1.0 cm below the sediment-water interface. The relative rate of $^{14}$C-acetate incorporation into PLFA per unit biomass (a measure of microbial growth; Findlay et al. 1985) was calculated using the average μmoles LPO4 for the appropriate time and treatment.

The rate of methyl-($^3$H)-thymidine incorporation into DNA was determined using the method of Moriarty & Pollard (1982). Sediments were extruded from the cores into plastic weighing dishes and mixed with a spatula. Approximately 1.5 g wet weight of the mixed sediments were placed in 50 ml plastic screw-cap test tubes with 2 ml of filtered seawater containing 65 μCi (50 Ci mmol$^{-1}$) of methyl-($^3$H)-thymidine. The test tubes were shaken and the samples incubated for 20 min. The incubations were stopped and the samples preserved

by adding 5 ml of a solution of 70 % ethanol and 5 % formaldehyde in 25 ppt artificial seawater. The samples were transported to the laboratory on ice and the labelled DNA was extracted. Calculations of methyl-($^3$H)-thymidine incorporation into DNA per unit biomass were made using the average μmoles LPO4 per g dry weight of sediment for the appropriate time and treatment.

**Statistical analysis.** The sieving disturbance experiment was designed to be descriptive. Four t-tests were used to facilitate discussion of changes observed among several of the sampling times. These comparisons were performed to determine if sieved sediments were significantly different from ambient sediments, and if the first 2 changes following sieving were significant. Specifically, for measures that declined following sieving, the 4 tests were: (1) the value for ambient sediments was compared to the value for sieved sediments, (2) the value for ambient sediments was compared to the first minimum value, (3) the first minimum value was compared to the following relative maximum value, and, (4) values for the ambient sediments and the relative maximum were compared. For measures that increased following sieving, the 4 tests were: (1) the value for ambient sediments was compared to the value for sieved sediments, (2) the value for ambient sediments was compared to the first maximum value, (3) the first maximum value was compared to the following relative minimum value, and, (4) values for the ambient sediments and the relative minimum were compared. Data were natural log transformed prior to statistical analysis.

Groups of phospholipid fatty acids appeared to have similar patterns of change over the course of the experiment. An a posteriori cluster analysis was used to confirm these groupings. Fatty-acids profiles (Appendix 1) were transformed by dividing the nmoles of fatty acid measured at each sampling time by the maximum nmoles of that fatty acid recorded during the experiment. This allowed analysis of the patterns of change independent of the absolute values of the data (Pielou 1984). Cluster analysis was performed using the cluster sub-program available in Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA). The squared Euclidean measure of distances and average linkage between groups were utilized.

## RESULTS

Short-term metabolic status, measured using the ratio 14CPLFA/14CPHA, was significantly higher immediately following sieving, obtained a maximum value 2 h after the disturbance, and returned to ambient values 8 h after the sediments were sieved (Table 1, Fig. 1A). This ratio continued to decline to a

Table 1. Effect of sieving on microbial activity and short-term metabolic status. All data given as means ± SD, n = 3. PLFA: phospholipid fatty acids; PHA: poly-beta-hydroxyalkanoates; LPO4: total phospholipid phosphate

| Time (h) after sieving | DPM's PLFA/ DPM's PHA | pmol thymidine incorporated into DNA $min^{-1}$ $\mu mol^{-1}$ LPO4 | nmol acetate incorporated into phospholipid $min^{-1}$ $\mu mol^{-1}$ LPO4 |
|---|---|---|---|
| Ambient sediment | 0.37 ± 0.04 | 2.13 ± 0.40 | 2.56 ± 1.50 |
| Sieved sediment | 0.53 ± 0.02 | 0.94 ± 0.12 | 0.56 ± 0.16 |
| 2 | 0.61 ± 0.10 | 1.29 ± 0.43 | 1.09 ± 0.31 |
| 4 | 0.57 ± 0.10 | 1.54 ± 0.46 | 0.53 ± 0.11 |
| 8 | 0.42 ± 0.08 | 1.39 ± 0.33 | 0.54 ± 0.13 |
| 12 | 0.39 ± 0.07 | 1.40 ± 0.26 | 0.52 ± 0.18 |
| 24 | 0.39 ± 0.14 | 1.43 ± 0.43 | 0.64 ± 0.27 |
| 48 | 0.27 ± 0.06 | 1.74 ± 1.21 | 0.54 ± 0.13 |
| 72 | 0.18 ± 0.05 | 1.61 ± 0.52 | 0.47 ± 0.07 |
| 120 | 0.39 ± 0.04 | 1.33 ± 0.22 | 0.74 ± 0.12 |

Statistical summary[a]

| PLFA/PHA | Significance | ^{3}H-thymidine | Significance | ^{14}C-acetate | Significance |
|---|---|---|---|---|---|
| Ambient vs sieved | $p < 0.01$ | Ambient vs sieved | $p < 0.01$ | Ambient vs sieved | $p < 0.05$ |
| Ambient vs 1st max. (2 h) | $p < 0.05$ | Ambient vs 1st min. (sv) | $p < 0.01$ | Ambient vs 1st min. (sv) | $p < 0.05$ |
| 1st max. vs rel. min. (12 h) | $p < 0.05$ | 1st min. vs rel. max. (4 h) | $p < 0.05$ | 1st min. vs rel. max. (2 h) | $p < 0.05$ |
| Ambient vs rel. min. | ns | Ambient vs rel. max. | ns | Ambient vs rel. max. | ns |

[a] Data natural log transformed before statistical analysis. Number in brackets following max. or min. indicates hours after sieving; sv indicates sieved sediments. ns: not significant

minimum value 3 d after sieving of the sediments and then increased to ambient levels at the end of the experiment.

Both measures of microbial growth were significantly lower immediately after sieving and then increased 2 h after the disturbance (Table 1, Fig. 1B, C). Growth rates, as measured as acetate incorporation into phospholipid, peaked 2 h after sieving while those measured as thymidine incorporation into DNA were highest 4 h after the disturbance. Growth rates throughout the rest of the experiment were lower than observed in the ambient sediment.

The 3 measures of microbial biomass were initially unchanged by sieving but then decreased to a minimum at either 2 h (LPO4) or 4 h (TPLFA, MA) after sieving (Table 2, Fig. 2). The decrease was greatest in TPLFA (77 %), moderate in MA (40 %), and smallest in LPO4 (18 %). All 3 measures then increased to levels approaching ambient values 8 and 12 h following the disturbance. Values for TPLFA, MA and LPO4 then remained essentially unchanged for the remainder of the experiment.

The amount of PHA, a measure of long-term metabolic status, recovered from the sediments was initially unchanged by sieving then decreased to zero 8 h after the disturbance. The recovery of this procaryotic storage material was much slower than the recovery of microbial biomass with values returning to

ambient levels 24 h after sieving (Table 3, Fig. 3). Levels then decreased to ca 20 % of those for ambient sediments for the remainder of the experiment. The trans/cis ratios for the fatty acids 16:1ω7 and 18:1ω7, a measure of stress or starvation, were unchanged by sieving but increased to a maximum value 4 h following the disturbance and returned to ambient values after 8 and 12 h (Table 3, Fig. 3). This pattern is virtually the opposite of the pattern observed for TPLFA. The ratios again increased and remained elevated for the remainder of the experiment.

Twenty-five individual PLFA were identified (Appendix 1). All fatty acids, as with TPLFA, were initially unaffected by sieving but then decreased in abundance to a minimum value 4 h after the disturbance. At 8 h after sieving the abundance of all the fatty acids had significantly increased. Cluster analysis revealed that the patterns of change in the individual fatty acids grouped into 4 distinct clusters (Fig. 4). A functional-group approach was utilized in the analysis of these patterns. Pure culture studies, laboratory manipulations on bacterial respiratory types, and descriptive field studies were the basis for assigning functional groups of microorganisms to the clusters obtained (see Appendix 2).

The fatty acids of Cluster 1, the microeucaryotic functional group, decreased following sieving with abundances falling to zero 4 h after the construction of the microcosms (Fig. 5, Appendix 1). These fatty acids

**A**



SHORT-TERM METABOLIC STATUS
(14CPLFA/14CPHA)

**B**



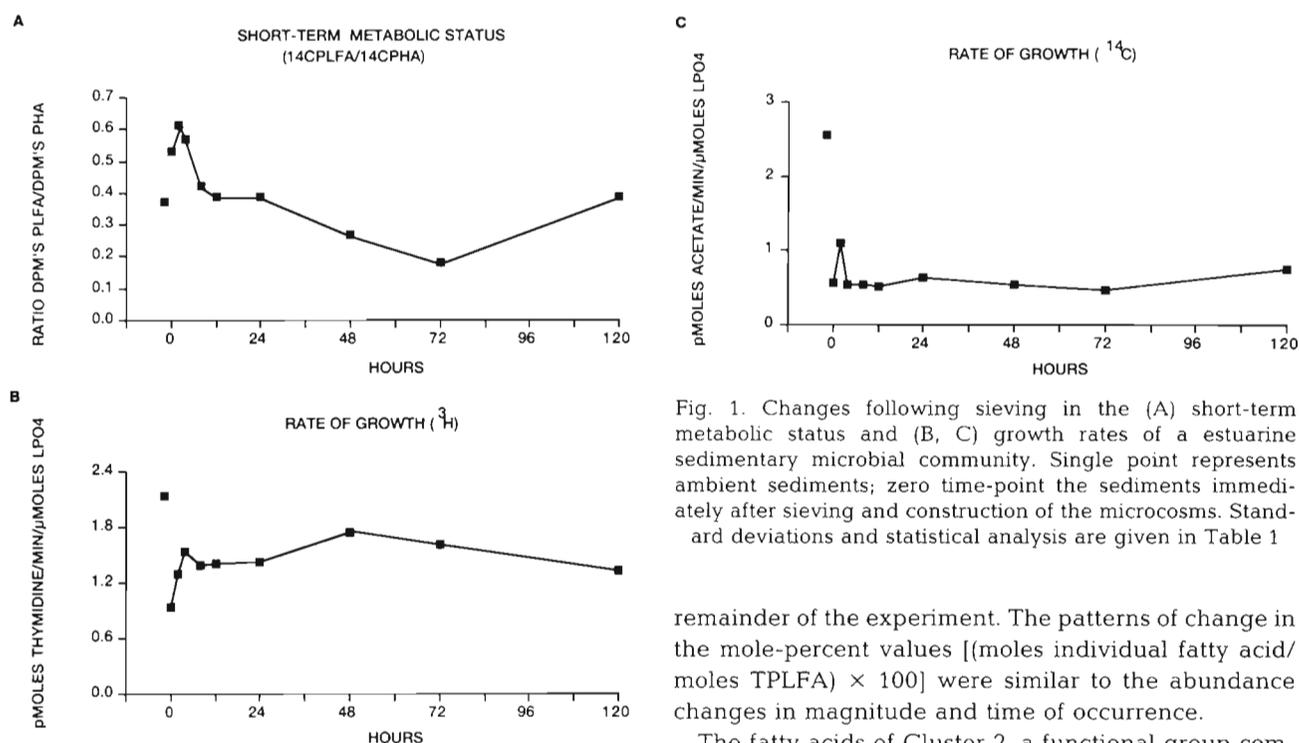RATE OF GROWTH ($^3$H)

**C**



RATE OF GROWTH ($^{14}$C)

Fig. 1. Changes following sieving in the (A) short-term metabolic status and (B, C) growth rates of a estuarine sedimentary microbial community. Single point represents ambient sediments; zero time-point the sediments immediately after sieving and construction of the microcosms. Standard deviations and statistical analysis are given in Table 1

increased moderately 8 h following sieving and peak abundances occurred 24 h after the disturbance. Abundances again declined 48 h after sieving and remained very low, compared to ambient sediments, for the

remainder of the experiment. The patterns of change in the mole-percent values [(moles individual fatty acid/ moles TPLFA) × 100] were similar to the abundance changes in magnitude and time of occurrence.

The fatty acids of Cluster 2, a functional group composed of aerobic procaryotes and eucaryotes, also decreased 4 h after sieving with abundances falling to less than 10 % of the levels in the ambient sediments. At 8 and 12 h after sieving the abundance of these fatty acids increased, but only between 50 and 75 % of the

Table 2. Effect of sieving on microbial biomass. All data given as μmol g$^{-1}$ dry wt of sediment; means ± SD, n = 3

| Time (h) after sieving | Total ester-linked, phospho- lipid fatty acids (TPLFA) | Total phospholipid phosphate | Muramic acid |
|---|---|---|---|
| Ambient sediment | 19.3 ± 5.8 | 52.5 ± 5.7 | 21.2 ± 8.2 |
| Sieved sediment | 15.1 ± 5.3 | 48.4 ± 2.3 | 14.8 ± 4.0 |
| 2 | 11.6 ± 3.2 | 44.6 ± 1.9 | 13.1 ± 3.6 |
| 4 | 4.5 ± 3.6 | 46.2 ± 3.9 | 12.7 ± 8.0 |
| 8 | 13.0 ± 3.3 | 51.5 ± 5.6 | 15.3 ± 3.2 |
| 12 | 12.9 ± 4.8 | 49.2 ± 6.8 | 13.6 ± 3.4 |
| 24 | 7.8 ± 2.9 | 52.4 ± 13.6 | 14.5 ± 4.4 |
| 48 | 5.7 ± 3.7 | 49.0 ± 16.3 | 13.6 ± 4.3 |
| 72 | 11.2 ± 5.9 | 53.0 ± 2.4 | 16.6 ± 6.4 |
| 120 | 14.6 ± 2.0 | 47.0 ± 3.9 | 17.3 ± 1.5 |

Statistical summary[a]

| TPLFA | Significance | LPO4 | Significance | Muramic acid | Significance |
|---|---|---|---|---|---|
| Ambient vs sieved | ns | Ambient vs sieved | ns | Ambient vs sieved | ns |
| Ambient vs 1st min. (4 h) | $p < 0.02$ | Ambient vs 1st min. (2 h) | $p < 0.05$ | Ambient vs 1st min. (4 h) | $p < 0.1$ |
| 1st min. vs rel. max. (8 h) | $p < 0.05$ | 1st min. vs rel. max. (8 h) | $p < 0.05$ | 1st min. vs rel. max. (8 h) | ns |
| Ambient vs rel. max. | ns | Ambient vs rel. max. | ns | Ambient vs rel. max. | ns |

[a] Data natural log transformed before statistical analysis. Number in brackets following max. or min. indicates hours after sieving. ns: not significant
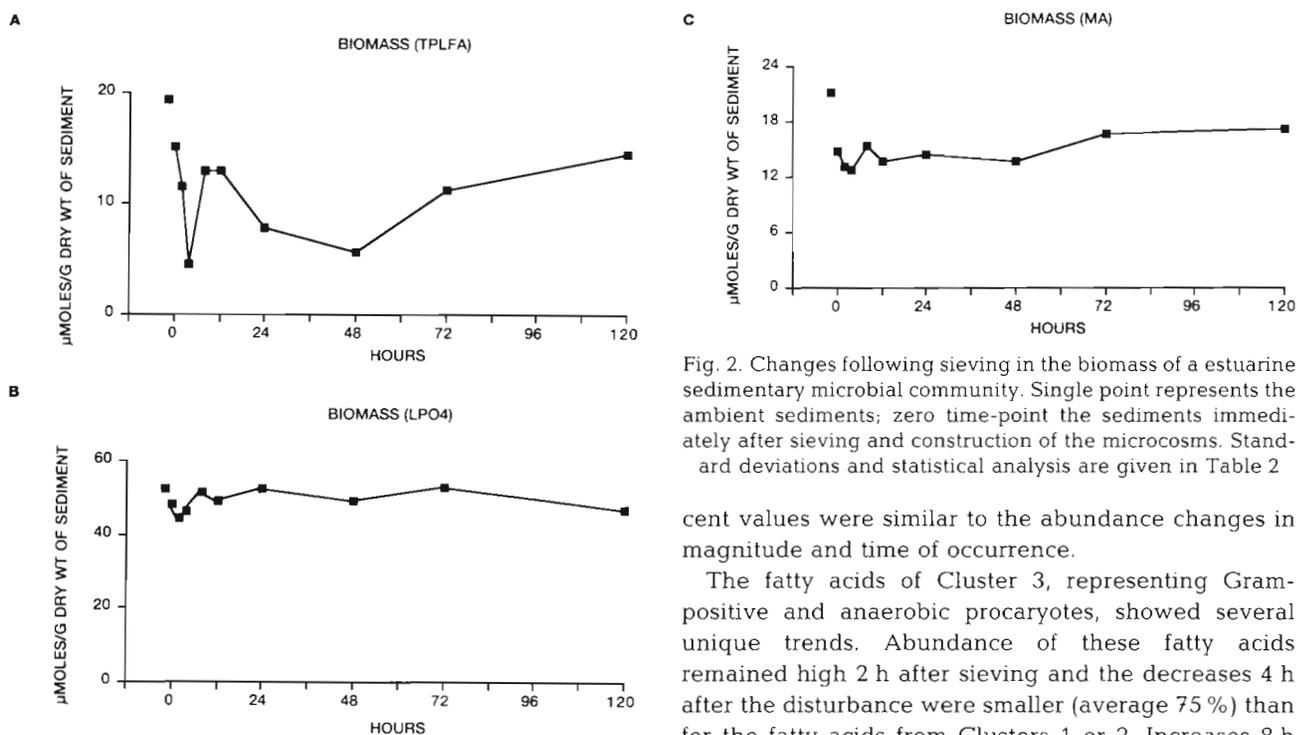
**A**

BIOMASS (TPLFA)



**B**

BIOMASS (LPO4)



**C**

BIOMASS (MA)



Fig. 2. Changes following sieving in the biomass of a estuarine sedimentary microbial community. Single point represents the ambient sediments; zero time-point the sediments immediately after sieving and construction of the microcosms. Standard deviations and statistical analysis are given in Table 2

cent values were similar to the abundance changes in magnitude and time of occurrence.

The fatty acids of Cluster 3, representing Gram-positive and anaerobic procaryotes, showed several unique trends. Abundance of these fatty acids remained high 2 h after sieving and the decreases 4 h after the disturbance were smaller (average 75 %) than for the fatty acids from Clusters 1 or 2. Increases 8 h after sieving were similar to those observed in Cluster 2. At the end of the experiment, the abundance and mole-percent values of Cluster 3 fatty acids were comparable to those of the ambient sediment.

levels in the ambient sediments. The fatty acids again decreased in abundance 24 h after sieving and then remained fairly constant for the remainder of the experiment. The patterns of change in the mole-per-

Table 3. Effect of sieving on long-term microbial metabolic status. All data given as means $\pm$ SD, n = 3; PHA as nmol g$^{-1}$ dry wt of sediment

| Time (h) after sieving | PHA | 16:1$\omega$7 trans/cis isomer ratio | 18:1$\omega$7 trans/cis isomer ratio |
|---|---|---|---|
| Ambient sediment | 2275 $\pm$ 417 | 0.080 $\pm$ 0.011 | 0.021 $\pm$ 0.008 |
| Sieved sediment | 1113 $\pm$ 1733 | 0.081 $\pm$ 0.017 | 0.022 $\pm$ 0.008 |
| 2 | 177 $\pm$ 306 | 0.165 $\pm$ 0.080 | 0.043 $\pm$ 0.013 |
| 4 | 23 $\pm$ 28 | 0.905 $\pm$ 0.774 | 0.057 $\pm$ 0.031 |
| 8 | 0 $\pm$ 0 | 0.116 $\pm$ 0.039 | 0.034 $\pm$ 0.029 |
| 12 | 453 $\pm$ 582 | 0.087 $\pm$ 0.006 | 0.018 $\pm$ 0.006 |
| 24 | 2507 $\pm$ 1708 | 0.241 $\pm$ 0.211 | 0.021 $\pm$ 0.015 |
| 48 | 577 $\pm$ 230 | 0.302 $\pm$ 0.188 | 0.054 $\pm$ 0.003 |
| 72 | 225 $\pm$ 148 | 0.240 $\pm$ 0.104 | 0.037 $\pm$ 0.025 |
| 120 | 285 $\pm$ 120 | 0.350 $\pm$ 0.189 | 0.043 $\pm$ 0.010 |

Statistical summary[a]

| PHA | Significance | 16:$\omega$7 t/c ratio | Significance | 18:1$\omega$7 t/c ratio | Significance |
|---|---|---|---|---|---|
| Ambient vs sieved | ns | Ambient vs sieved | ns | Ambient vs sieved | ns |
| Ambient vs 1st min. (8 h) | p < 0.01 | Ambient vs 1st max. (4 h) | p < 0.05 | Ambient vs 1st max. (4 h) | p < 0.05 |
| 1st min. vs rel. max. (24 h) | p < 0.05 | 1st max. vs rel. min. (12 h) | p < 0.05 | 1st min. vs rel. min. (12 h) | p < 0.05 |
| Ambient vs rel. max. | ns | Ambient vs rel. min. | ns | Ambient vs rel. max. | ns |

[a] Data natural log transformed before statistical analysis. Number in brackets following max. or min. indicates hours after sieving. ns: not significant
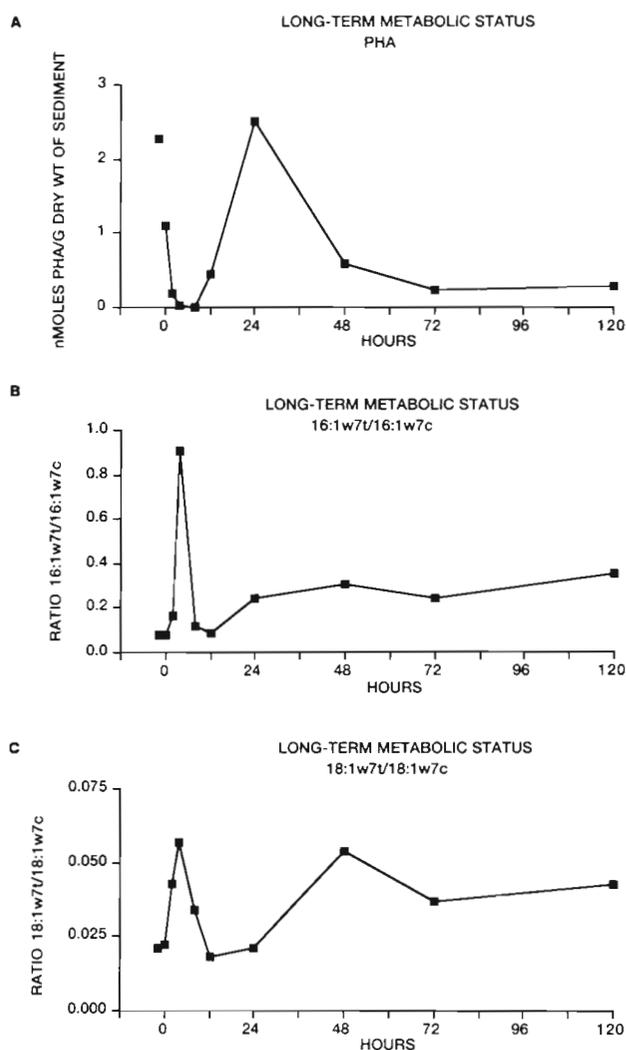
**A**

LONG-TERM METABOLIC STATUS
PHA



**B**

LONG-TERM METABOLIC STATUS
16:1w7t/16:1w7c



**C**

LONG-TERM METABOLIC STATUS
18:1w7t/18:1w7c

Fig. 3. Changes following sieving in the long-term metabolic status of a estuarine sedimentary microbial community. Single point represents the ambient sediments; zero time-point the sediments immediately after sieving and construction of the microcosms. Standard deviations and statistical analysis are given in Table 3

The fatty acids of Cluster 4, a functional group made up of sulfate-reducing bacteria and other anaerobic procaryotes, showed the smallest percentage decrease abundance 4 h after sieving. The decreases ranged from 33 to 50 %. The abundance of Cluster 4 fatty acids increased 8 h after sieving to a level comparable to the ambient sediments. At the end of the experiment the abundance of Cluster 4 fatty acids was greater in the microcosm sediments than in ambient sediments. The mole-percent values for each of these fatty acids increased to a maximum 4 h after sieving and then decreased at 8 and 12 h. After 5 d in the laboratory, the mole-percent values of the Cluster 4 fatty acids were greater in the microcosm sediments than in ambient sediments.
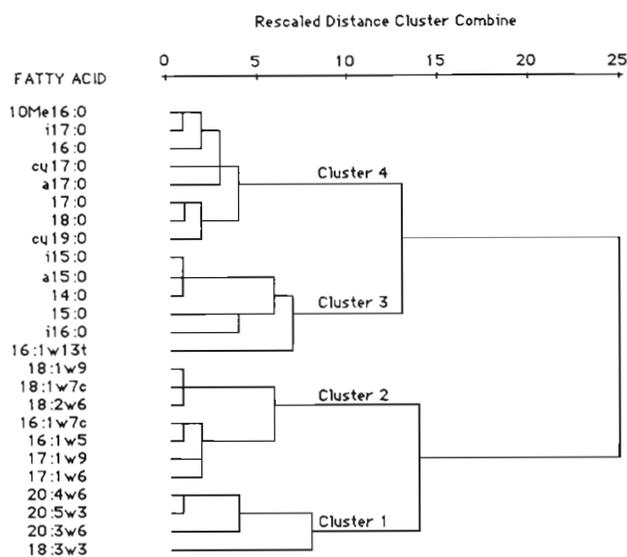


Fig. 4. Cluster analysis of the pattern of changes in the individual ester-linked, phospholipid fatty acids following sieving

## DISCUSSION

The effects of sieving on the microbial community varied with time following the disturbance. Immediately following sieving, the ratio of acetate-incorporation into phospholipid versus acetate-incorporation into PHA indicated that short-term metabolic status was shifted towards phospholipid synthesis. In addition, microbial growth rates were depressed and microbial biomass was unchanged. At 2 and 4 h after the disturbance, short-term metabolic status was still shifted towards phospholipid synthesis and growth rates were comparable to those of the ambient sediments. Microbial biomass was now significantly less in the disturbed sediments. Long-term measures of metabolic status (PHA, trans/cis ratios) indicate that the community was undergoing metabolic stress, possibly starvation or anoxia (Findlay & White 1983b, Guckert et al. 1985, 1986). Differential changes in the mole-percent values of the individual ester-linked, phospholipid fatty acids indicate that the relative importance of some functional groups within the microbial community had increased while others had declined. At 8 and 12 h after the disturbance the effects were minimal. The short-term metabolic status and total microbial biomass were comparable to the ambient sediments. The microbial community structure was once again similar to that of the ambient sediments and markedly different from the community several hours earlier. The levels of PHA were beginning to recover and the trans/cis ratios were again similar to those of the ambient sediments. Only microbial growth rates were significantly lower.

Sieving was initially deleterious. The effects were an immediate reduction in microbial growth rates,
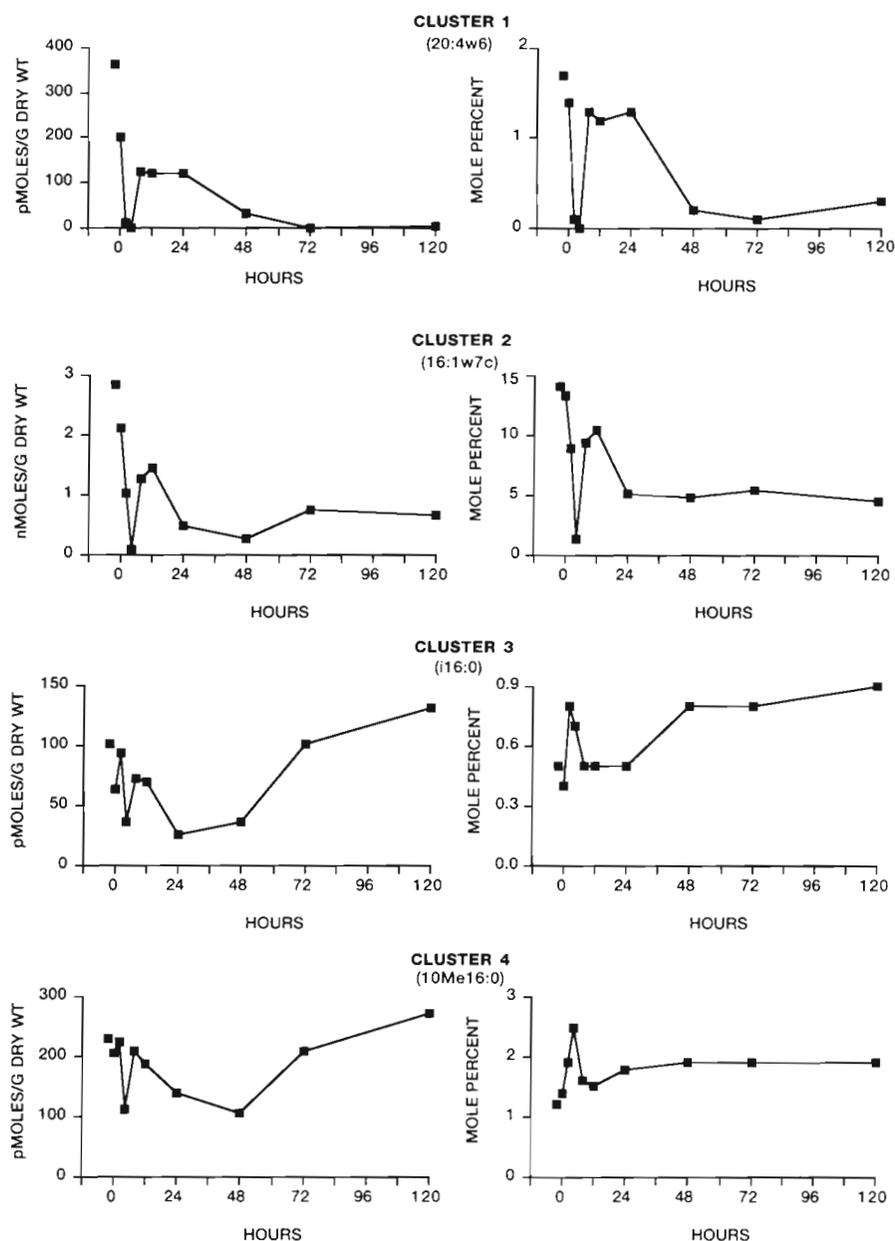
Fig. 5. Example of the pattern of changes in the abundance and mole-percent values of Cluster 1, 2, 3, 4 fatty acids (20:4ω6, 16:1ω7c, i16:0, 10Me16:0, respectively; from Appendix 1) induced by sieving of the sediments and their maintenance in laboratory microcosms

and a decrease in microbial biomass and storage products, and were also evidenced by the increase in the *trans/cis* fatty acid ratios. Sieving presumably abrades sediments and has been shown to transform heterogeneous sediments containing numerous complex biological and chemical gradients to a homogeneous mixture (Aller & Yingst 1985). Physical abrasion has been proposed to restrict microorganisms to the protected areas of sand grains (Meadows & Anderson 1967). Hoppe (1984) viewed attachment within protected sites of a sand grain as necessary for microbe survival in areas of high mechanical disturbance. Sediments with surface irregularities support a greater microbial biomass than those having smooth

surfaces (Nickels et al. 1981) and microscopic analysis of distribution patterns indicates that microbes are not uniformly distributed over the surfaces of sand grains, but are concentrated in cracks, crevices, and pits (Weise & Rheinheimer 1978). The initial deleterious effects observed following sieving may arise from abrasion and/or the exposure of the microorganisms to unfavorable metabolic conditions caused by the disruption of the gradients commonly associated with estuarine sediments.

The microbial community, first adversely affected by sieving, rapidly showed signs of recovery. Short-term metabolic status was immediately shifted towards cellular growth. Microbial growth increased several hours

after sieving and microbial biomass was comparable to that of ambient sediments after 8 h. It is widely argued that bacteria in nature experience restricted rates of growth due to limitation of essential resources (e.g. Tempest & Neijssel 1978, Gottschal 1985). The stimulatory effects of disturbance may arise by a reduction of resource limitation; specifically, by opening space for microbial colonization and growth, by increasing nutrient availability, and by decreasing the concentration of inhibitory metabolites.

The differential responses of the various fatty-acid clusters following sieving indicated the functional groups within the microbial community varied in their response to the disturbance. The large decrease in abundance of Clusters 1 and 2 fatty acids 4 h after sieving indicates that aerobic organisms, both eucaryotic and procaryotic, were severely affected. The drop in mole-percent values of these fatty acids indicated a disproportionate negative effect on the aerobic organisms. The large increase in the *trans/cis* ratios of fatty acids indicates aerobic organisms were under stressful metabolic conditions at this time (Guckert et al. 1986). The moderate decrease in Cluster 4 fatty acids indicates that *Desulfobacter* and other anaerobic bacteria were only moderately affected. The increase in mole-percent values 4 h after sieving indicates that this group of organisms increased in relative importance within the community. The presence of mixed micro-colonies (Hirsch 1984, White 1984), composed of an outer layer of aerobes more susceptible to abrasion, and an inner, more protected core of anaerobes would account for a differential loss of aerobic microorganisms.

All 4 groups of organisms rapidly recovered from the adverse effects of sieving. The 3 functional groups dominated by bacteria (2, 3 and 4) showed significant increases in biomass 8 h after the disturbance. The recovery of microeucaryotic biomass, as indicated by the abundance of Cluster 1 fatty acids, was slower than the recovery of procaryotic biomass, with peak eucaryotic biomass occurring 24 h after the disturbance. Mole-percent values indicated that the relative abundance of microeucaryotes at this time was similar to ambient relative abundances.

The microcosm sediments failed to develop the thin layer of oxidized sediment present in the natural environment. After 5 d, Cluster 1 fatty acids had significantly decreased indicating a loss of microeucaryotic biomass. Clusters 3 and 4 fatty acids increased relative to Cluster 2 fatty acids indicating a relative increase in anaerobic organisms compared to aerobic organisms. PHA abundances decreased and the *trans/cis* ratios increased indicating aerobic microbes were under metabolic stress. These changes in microbial community structure are similar to those reported by Federle

and co-workers (1983a, 1983b, 1986). In a series of carefully controlled experiments they determined the microbial community structure of sediments at a nearby site in St. Georges Sound and the effects of maintaining these sediments in the laboratory. Microcosms were carefully constructed to maintain sediment integrity and minimize disturbance. In situ light, salinity, temperature and dissolved oxygen were controlled. After 2 wk, a discriminant analysis indicated that microcosm sediments were depauprate in 16:1ω7 and 18:2ω6 (Cluster 2 fatty acids, this study) and enriched in 15:0 (Cluster 3 fatty acids, this study) when compared to field control sediments.

Near-shore and intertidal sediments are exposed to significant wave and tidal action. Wave-generated hydrostatic pressure has been shown to increase water-soluble transport, increasing oxygen penetration into sediments (Steele et al. 1970, Riedl et al. 1972, Webb & Theodor 1972). The combined effects of reduced circulation within the sediment and the loss of controlling biotic factors may have induced the changes observed in the microcosm sediments after 5 d. Reduced availability of oxygen in the sediments may have caused the loss of microeucaryotic biomass, the decrease in aerobic bacteria and the increase in sulfate-reducing and other anaerobic bacteria. The decrease in abundance of PHA without an increase in the growth rate or a shift in short-term metabolic status toward cellular growth suggests that carbon and energy had become limiting. The rise in the *trans/cis* fatty acid ratio supports this conclusion. This may be caused by the loss of detrital carbon inputs from overlying waters and/or decreased rates of flux of reduced carbon from depth. The reduced rates of growth may also be a consequence of reduced availability of carbon and energy.

The results of our study, in agreement with the conclusions of Federle et al. (1986), suggest that care should be taken when designing a microcosm study, especially sieved-sediment microcosms commonly used to study macrofaunal-microbial interactions. Sediments should be characterized prior to microcosm construction to allow assessment of changes induced during construction. In addition, experimental designs should allow for detection of changes arising from uncoupling the sedimentary community from biotic and abiotic controls.

In summary, the dramatic changes observed in the microbial community in the 24 h following sieving demonstrates the potential for disturbance playing an important role in structuring microbial communities in marine sediments. In addition, the changes noted after sediments were maintained in the laboratory for 5 d suggest that abiotic factors that increase oxygen penetration into the sediments also influence microbial community structure.

**Appendix 1.** Fatty-acid profiles from ambient sediments, sieved sediments and microcosm sediments 2 through 120 h after sieving. Data are nmol of ester-linked, phospholid fatty acids g⁻¹ dry wt sediment, given as means ± SD, n = 3

| Fatty acid | Ambient sediment | Sieved sediment | Microcosm sediments – hours after sieving | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | 12 | 24 | 48 | 72 | 120 |
| **Cluster 1** | | | | | | | | | | |
| 18:3ω3 | 24 ± 29 | 52 ± 77 | 0 ± 0 | 0 ± 0 | 7 ± 12 | 3 ± 5 | 22 ± 26 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 20:3ω6 | 43 ± 27 | 41 ± 15 | 12 ± 20 | 0 ± 0 | 21 ± 21 | 24 ± 14 | 24 ± 14 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 20:4ω6 | 362 ± 303 | 204 ± 74 | 11 ± 19 | 0 ± 0 | 124 ± 145 | 122 ± 64 | 122 ± 146 | 31 ± 54 | 1 ± 2 | 3 ± 5 |
| 20:5ω3 | 128 ± 109 | 84 ± 73 | 9 ± 16 | 0 ± 0 | 41 ± 71 | 39 ± 39 | 76 ± 98 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **Cluster 2** | | | | | | | | | | |
| 16:1ω5 | 56 ± 30 | 43 ± 25 | 26 ± 4 | 0 ± 0 | 27 ± 17 | 28 ± 24 | 9 ± 10 | 5 ± 8 | 15 ± 26 | 13 ± 22 |
| 16:1ω7c | 2835 ± 1513 | 2112 ± 1121 | 1029 ± 327 | 90 ± 140 | 1259 ± 485 | 1458 ± 803 | 477 ± 498 | 260 ± 225 | 759 ± 798 | 666 ± 386 |
| 17:1ω6 | 181 ± 69 | 157 ± 47 | 73 ± 54 | 0 ± 0 | 82 ± 35 | 88 ± 53 | 33 ± 38 | 31 ± 32 | 40 ± 69 | 23 ± 39 |
| 17:1ω9 | 567 ± 223 | 514 ± 173 | 193 ± 84 | 19 ± 26 | 329 ± 27 | 336 ± 121 | 178 ± 179 | 99 ± 68 | 158 ± 149 | 149 ± 155 |
| 18:1ω7c | 3006 ± 1047 | 2840 ± 708 | 1211 ± 528 | 257 ± 241 | 2126 ± 289 | 2236 ± 129 | 1271 ± 1022 | 768 ± 494 | 1030 ± 780 | 1200 ± 875 |
| 18:1ω9 | 498 ± 166 | 462 ± 86 | 177 ± 74 | 25 ± 31 | 358 ± 54 | 346 ± 76 | 233 ± 233 | 145 ± 103 | 168 ± 148 | 173 ± 231 |
| 18:2ω6 | 276 ± 109 | 187 ± 49 | 107 ± 63 | 25 ± 20 | 181 ± 26 | 207 ± 17 | 125 ± 127 | 66 ± 33 | 78 ± 52 | 80 ± 53 |
| **Cluster 3** | | | | | | | | | | |
| 14:0 | 198 ± 180 | 140 ± 183 | 232 ± 164 | 17 ± 18 | 74 ± 78 | 102 ± 103 | 17 ± 18 | 18 ± 18 | 231 ± 216 | 221 ± 166 |
| 15:0 | 646 ± 274 | 438 ± 309 | 489 ± 358 | 128 ± 160 | 361 ± 283 | 376 ± 291 | 107 ± 47 | 92 ± 72 | 542 ± 443 | 589 ± 314 |
| a15:0 | 201 ± 122 | 158 ± 156 | 206 ± 159 | 26 ± 39 | 115 ± 102 | 124 ± 112 | 22 ± 4 | 15 ± 14 | 199 ± 169 | 247 ± 183 |
| i15:0 | 163 ± 96 | 134 ± 132 | 182 ± 135 | 24 ± 38 | 104 ± 86 | 105 ± 96 | 18 ± 4 | 15 ± 15 | 194 ± 166 | 250 ± 189 |
| i16:0 | 102 ± 34 | 64 ± 37 | 94 ± 40 | 36 ± 36 | 72 ± 46 | 69 ± 38 | 26 ± 23 | 37 ± 14 | 101 ± 75 | 132 ± 43 |
| 16:1ω13t | 128 ± 46 | 100 ± 38 | 175 ± 109 | 30 ± 24 | 87 ± 34 | 54 ± 52 | 32 ± 20 | 32 ± 12 | 89 ± 67 | 94 ± 55 |
| **Cluster 4** | | | | | | | | | | |
| 16:0 | 7639 ± 1189 | 5436 ± 2179 | 5777 ± 1953 | 2772 ± 2401 | 5724 ± 2575 | 5494 ± 2674 | 4379 ± 895 | 2816 ± 2292 | 5623 ± 2990 | 8253 ± 1764 |
| 10Me16:0 | 229 ± 32 | 205 ± 52 | 224 ± 51 | 111 ± 81 | 208 ± 70 | 189 ± 60 | 140 ± 35 | 107 ± 60 | 209 ± 96 | 274 ± 48 |
| a17:0 | 124 ± 19 | 107 ± 20 | 89 ± 38 | 58 ± 32 | 111 ± 34 | 63 ± 59 | 78 ± 14 | 58 ± 34 | 114 ± 39 | 163 ± 46 |
| i17:0 | 136 ± 36 | 115 ± 28 | 128 ± 28 | 68 ± 42 | 118 ± 59 | 127 ± 40 | 106 ± 27 | 76 ± 41 | 136 ± 47 | 176 ± 16 |
| cy17:0 | 113 ± 24 | 92 ± 29 | 69 ± 30 | 49 ± 43 | 57 ± 38 | 51 ± 39 | 41 ± 21 | 53 ± 29 | 84 ± 36 | 97 ± 54 |
| 17:0 | 559 ± 72 | 486 ± 70 | 393 ± 120 | 282 ± 150 | 480 ± 115 | 445 ± 119 | 395 ± 81 | 299 ± 171 | 485 ± 133 | 610 ± 91 |
| 18:0 | 617 ± 53 | 580 ± 25 | 416 ± 93 | 368 ± 130 | 605 ± 98 | 570 ± 97 | 633 ± 113 | 501 ± 204 | 636 ± 148 | 832 ± 188 |
| cy19:0 | 102 ± 7 | 99 ± 6 | 54 ± 4 | 37 ± 21 | 90 ± 50 | 70 ± 9 | 52 ± 8 | 58 ± 20 | 71 ± 23 | 86 ± 57 |
| **TransPLFA[a]** | | | | | | | | | | |
| 16:1ω7t | 215 ± 83 | 157 ± 559 | 158 ± 73 | 42 ± 54 | 154 ± 96 | 128 ± 73 | 61 ± 23 | 58 ± 31 | 148 ± 112 | 186 ± 51 |
| 18:1ω7t | 66 ± 38 | 59 ± 8 | 25 ± 21 | 16 ± 21 | 67 ± 47 | 40 ± 18 | 19 ± 15 | 42 ± 29 | 44 ± 35 | 36 ± 19 |
| TPLFA[b] | 19300 ± 5800 | 15100 ± 5300 | 11600 ± 3200 | 4500 ± 3600 | 12000 ± 3300 | 12900 ± 4800 | 7800 ± 2900 | 5700 ± 3700 | 11200 ± 5900 | 14600 ± 2000 |

[a] Trans isomers not included in cluster analysis. Molar amount per cell highly variable with metabolic status of culture (see Guckert et al. 1985 for discussion)
[b] Mole-percent values can be estimated by |(mean individual fatty acid/mean TPLFA) × 100]. Standard deviations are slightly overestimated by this method

Appendix 2. Summary of literature used to define functional groups. Number in parentheses indicate the references used in the assignments. Fatty acids were grouped into clusters by statistical analysis and functional group designations were made using all available information. Note that fatty acids with broad phylogenetic distribution should not be considered 'universal biomarkers' for their assigned functional group

| Fatty acid | Phylogenetic distribution (from pure cultures) | Bacterial metabolic type (mixed sediment isolations) | Functional group [as per (1, 2)] | Functional group (present study) |
|---|---|---|---|---|
| **Cluster 1** | | | | |
| 18:3ω3 | Microeucaryotes (3, 4); marine algae (5); fungi (6) | | Microeucaryotes | Microeucaryotes |
| 20:3ω6 | Microeucaryotes (3, 4, 7) | | Microeucaryotes | |
| 20:4ω6 | Microeucaryotes (3, 4, 7, 8) | | Microeucaryotes | |
| 20:5ω3 | Microeucaryotes (3, 4, 7, 8); diatoms (9) | | Microeucaryotes | |
| **Cluster 2** | | | | |
| 16:1ω5 | Bacteria (10) | | | Aerobic procaryotes and eucaryotes |
| 16:1ω7c | Bacteria (10); marine microfauna (8) | Aerobic (21, 22) | | |
| 17:1ω6 | Bacteria (10) | | | |
| 17:1ω9 | Bacteria (10) | | | |
| 18:1ω7c | Bacteria (10) | Aerobic (21); sulfate red. (22) | Gram-negative procaryotes | |
| 18:1ω9 | Bacteria (10); marine microfauna (8) | | | |
| 18:2ω6 | Fungi (6, 11, 12); algae (5, 13); protozoa (7); cyanobacteria (14) | | | |
| **Cluster 3** | | | | |
| 14:0 | Broad | Anaerobic (21, 22) | | Gram-positive procaryotes and other anaerobic bacteria (excepting 16:1ω13t) |
| a15:0 | Bacteria (3, 4, 10, 15); Gram + bacteria (23); *Desulfovibrio* (16) | | Gram-positive procaryotes | |
| i15:0 | Bacteria (3, 4, 10, 15); Gram + bacteria (23); *Desulfovibrio* (16) | Sulfate-reducing (22) | Gram-positive procaryotes | |
| 15:0 | Broad | Anaerobic (21) | Procaryotes | |
| i16:0 | Bacteria (3, 4, 10, 15); *Desulfovibrio* (16) | | | |
| 16:1ω13t | Photosystem 1 (17) | | Photoautotrophs | |
| **Cluster 4** | | | | |
| 16:0 | Broad | | | Sulfate-reducing bacteria and other anaerobic procaryotes |
| 10Me16:0 | Bacteria (4); *Desulfobacter* (18) | | *Desulfobacter* | |
| a17:0 | Bacteria (4); Gram + bacteria (23); *Desulfovibrio* (16, 19, 20) | | Gram-positive procaryotes | |
| i17:0 | Bacteria (4); Gram + bacteria (23); *Desulfovibrio* (16, 19, 20) | | Gram-positive procaryotes | |
| cy17:0 | Bacteria (4); *Desulfobacter* (18) | Anaerobic (21) | *Desulfobacter* | |
| 17:0 | Broad | | Procaryotes | |
| 18:0 | Broad | | | |
| cy19:0 | Bacteria (4, 10) | Anaerobic (21) | Procaryotes | |

References cited in Appendix 2: (1) Dobbs & Guckert (1988a); (2) Dobbs & Guckert (1988b); (3) Bobbie & White (1980); (4) White (1983); (5) Wood (1974); (6) Fell & Findlay (1987); (7) Erwin (1973); (8) Findlay et al. (1986); (9) Gillian & Hogg (1984); (10) Gillian et al. (1983); (11) Wassef (1977); (12) Weete (1980); (13) Holz (1981); (14) Parker et al. (1967); (15) Volkman & Johns (1977); (16) Edlund et al. (1985); (17) Wannigama et al. (1981); (18) Dowling et al. (1986); (19) Boon et al. (1977); (20) Boon et al. (1978); (21) Guckert et al. (1985); (22) Parkes & Taylor (1983); (23) Kaneda (1977)

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