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Fatty acid composition and microbial activity of benthic marine sediment from McMurdo Sound, Antarctica

(Signature lipids; community structure; gas chromatography-mass spectrometry)

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1. SUMMARY

Signature lipids from the phospholipid ester-linked fatty acids (PELFA) of cell membranes were used to describe benthic microbial communities of 4 Antarctic sediments. Metabolic activities of the communities were determined by incorporation of [^{3}H]thymidine into bacterial DNA and sodium [^{14}C]acetate into membrane lipids. Biomass measurements from extractable phospholipid fatty acids per g dry wt. ranged between 6 to 76 nmol, or when converted to number of bacteria, 3.7×10^8 to 4.5×10^9 cells per g dry wt. The West Sound site at New Harbor contained the lowest biomass, while Cape Evans on the East Sound contained the greatest. A marked difference was also noted between sites in their sediment microbial community structure. The East Sound sites at Cape Armitage and Cape Evans contained a greater abundance of diatom marker lipids, whilst both sides of the Sound contained approximately the same relative amounts of bacterial groups distinguished using PELFA. Activity of sediment microorganisms measured by radiolabel incorporation under ambient conditions followed the trends of the biomass measurements. The East Sound sites were more active by an average of 45–73% for [^{3}H]thymidine and possibly also for sodium [^{14}C]acetate.

2. INTRODUCTION

Membrane lipids from viable cells have recently been used in many environments to describe and quantify microbial communities. Analysis of the methyl esters of fatty acids derived from the phospholipids of microorganisms associated with biofilms, soils and sediments provides a reproducible and quantitative measure of the biomass and community structure of microbial assemblies [1]. Other investigators have analysed fatty acids derived from the total lipid extract [1,2]. However, considerable information can be obtained by examining the fatty acids of individ-

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ual lipid classes within the total lipid, rather than studying the total lipid. Microbial biomass as determined by total phospholipid has been shown to correlate well with other measures of biomass (e.g., enzyme activities, muramic acid levels, total ATP, and respiratory activity) [3]. In addition, fatty acid methods have been proved to be free of distortions introduced with requirements for quantitative removal of organisms from surfaces, and the selectivity introduced when organisms are grown on artificial substrates [3]. Such chemical methods also avoid the difficulty and length of time involved in the isolation and culturing of psycrophilic organisms. These biochemical techniques have also been expanded and refined to describe those organisms associated with extreme environments. Microorganisms from such environments as geothermal vents [4,5], deep sea trenches [6] and deep subsurface aquifers [7], as well as halophilic [8,9] and methanogenic archaebacterial isolates [10], have been described by their membrane lipids.

These same techniques have most recently been utilized by the authors to identify microbial signature lipids of as yet undescribed Antarctic benthic marine microorganisms. In addition to signature lipids, metabolic activity was measured by incorporation of the radiolabels methyl[^{3}H]-thymidine into bacterial DNA and sodium [1-^{14}C]acetate into lipids. Microbial metabolic activity has been shown to be considerably lower in colder (5°C) marine sediments [11,12] than in warmer temperate regions. However, rates of assimilation of ^{3}H-ATP at 0°C into water column microorganisms up to a depth of 400 m on the East side of McMurdo Sound, Antarctica, were found to be similar to rates in temperate (15–20°C) eutrophic waters off La Jolla, California [13].

In this report the microbial signature lipids and metabolic activity of 4 different sediment sites from McMurdo Sound are reported. One goal of this study was to provide biomass estimates together with additional information on the microbial community structure. These data are presented to provide a step towards a broader global view of microorganisms in extreme environments and a base for future food-chain work in this unique environment.

3. MATERIALS AND METHODS

Solvents were all of residue analysis quality or better (Baker, Phillipsburg, NJ). Methyl[^{3}H]-thymidine (79.8 Ci/mmol) and sodium[1-^{14}C]acetate (45–60 mCi/mmol) were obtained from New England Nuclear (Boston, MA).

3.1. Sample collection

Sediment samples were collected from 4 sites in McMurdo Sound (Fig. 1). 3 sites were on the more productive East side of the Sound [14], with 2 sites at Cape Armitage — CA(1), 14.5 m; CA(2), 20 m, and one at Cape Evans — CE, 20 m. The fourth site, New Harbor — NH, 30.5 m, was a less productive area which has been suggested to resemble a deep sea sediment [14]. Duplicate sediment cores were collected at each site and were worked up separately as replicate samples. Ice diatom and *Phaeocystis* sp. algal blooms occur to varying degrees each austral summer in late December to early January, and are believed to be a major input of carbon to the benthos at certain Sound sites [15]. All collections were made before the algal blooms by divers at depths of 14.5 m to 30.5 m by hand coring. A summary of water column mean Antarctic physiochemical data for the Ross Sea area reported the following measurements: temperature, −1.87°C; salinity, 34.70‰; dissolved oxygen, 6.72 ml/l; pH, 7.84; phosphorus concentration, 1.82 μg atom/l; silica concentration, 44.8 μg atom/l [16]. Specific data for the sites sampled in this study were close to these reported values (Kottmeier, Sullivan and Kraft, personal communication). The under-ice and water column irradiance at a depth of 20 m was found to be between 0.6 and 35 $\mu E \cdot m^{-2} \cdot s^{-1}$. Care was taken to recover sediment intact without mixing the layers. This was done by inserting a 5 cm diameter core 10 cm into the sediment, placing a rubber stopper in the top and withdrawing the core from the sediment. Transportation to Eklund biological laboratory, sealed and in seawater at ambient temperature, was accomplished within 1–1.5 h of the time of coring. The cores were then extruded and the top 2 cm was washed through a 500 μm mesh sieve into a 250 ml stainless steel centrifuge can. This sieving served to prevent any

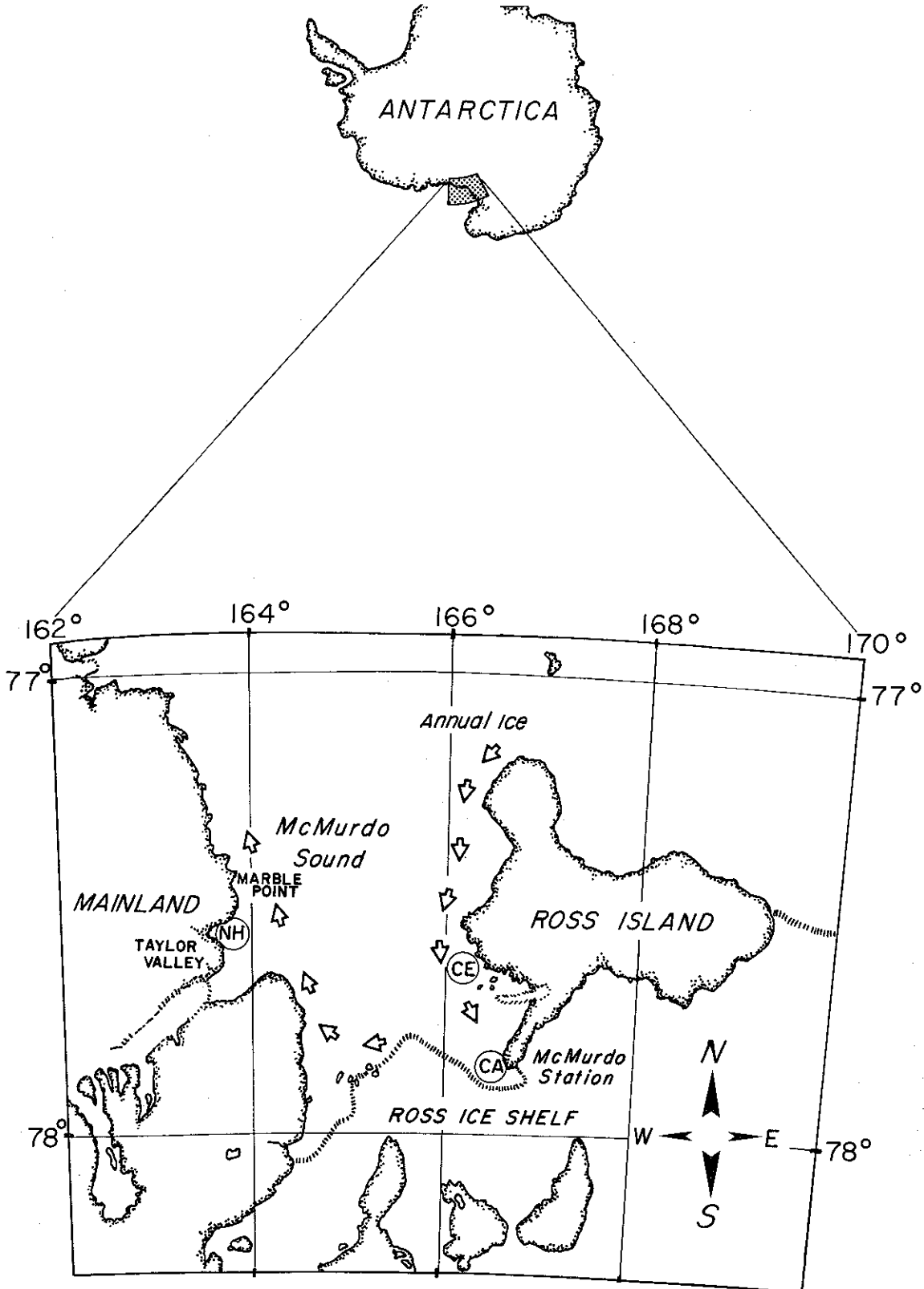

Fig. 1. McMurdo Sound, Antarctica, sampling sites. Cape Armitage (CA), Cape Evans (CE) and New Harbor (NH). Arrows indicate current direction.

large meiofauna from being extracted and biasing the lipid analysis of microorganisms.

3.2. Lipid extraction

The collected sediment was centrifuged at 10 000 × *g* for 10 min, the water was discarded and the sediment pellet extracted in the can using a single phase methanol : chloroform 2 : 1 (v/v) modification of the Bligh and Dyer [17] technique [18]. After allowing at least 12 h extraction time, the second phase solvents of chloroform : distilled water 1 : 1 (v/v), were added and mixed, then completely separated by centrifugation at 10 000 × *g* for 10 min and the supernatants transferred to a 250 ml separatory flask and allowed to separate once again. The lipid fraction was filtered through Whatman 2V filter paper into a 250 ml round-bottomed flask, and the solvent was removed using a rotary evaporator. The lipid was then transferred by chloroform washes into a screw-cap test tube, evaporated under a stream of nitrogen, capped and stored at −20°C for transport to Florida State University.

The dried lipid was transferred to a 1 g column of silicic acid (Unisil 100–200 mesh, Clarkson Chemical Co., Williamsport, PA.) and separated into neutral, glyco- and phospholipid fractions by consecutive 10-ml washes of chloroform, acetone and methanol [18]. The phospholipid fraction was dried under a stream of nitrogen, and the fatty acids were converted to methyl esters using a 0.2 M KOH in methanol solution [18]. Fatty acid methyl esters (FAME) were further separated by thin layer chromatography [19] into hydroxy and normal FAME.

Normal FAME samples were taken up in hexane with methylnonadecanoate (19 : 0) as the internal injection standard. Initial identification of individual normal FAME was performed by high resolution gas chromatography using a Hewlett Packard 5880A gas chromatograph equipped with a flame ionization detector. Samples were injected at 50°C in the splitless mode onto a non-polar cross-linked methyl silicone capillary column (50 m × 0.2 mm i.d., Hewlett Packard) using a Hewlett Packard 7672 automatic sampler. The oven was temperature programmed from 50°C to 160°C at 10°C per min, then at 2°C per min to 300°C. Hydrogen was used as the carrier gas. The injector and detector were maintained at 300°C.

Tentative peak identification, prior to GC–MS analysis, was based on comparison of retention times with those obtained for standards from Supelco (Bellefonte, PA) and Applied Science Laboratories (State College, PA) and previously GC–MS identified laboratory standards. Peak areas were quantified using a Hewlett Packard 3350 series programmable laboratory data system operated in an internal standard program. Fatty acid compositional data reported for these samples are expressed as average mol% of the total molar quantity for each replicate sediment core ($N = 2$, 1 SD) and are therefore relative.

3.3 Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed on a Hewlett Packard 5996A system fitted with a direct capillary inlet. The same column type as described above was used. Samples were injected in the splitless mode at 100°C with a 0.5 min venting time after which the oven was temperature programmed to 300°C at either 3 or 4°C per min. Helium was used as the carrier gas. MS operating parameters were: electron multiplier between 1 300–1 400 V, transfer line 300°C, source and analyzer 250°C, autotune file DFTPP normalized, optics tuned at *m/z* 502, electron impact energy = 70 eV. Mass spectral data were acquired and processed using a Hewlett Packard RTE-6/VM data system.

The dimethyldisulfide (DMDS) adducts of monounsaturated FAME were formed using the method described by Dunkelblum et al. [20] to locate the double bond positions (Table 1). Samples in hexane (50 µl) were treated with 100 µl DMDS (Gold Label, Aldrich Chemical Co., Milwaukee, WI) and 1–2 drops of iodine solution (6.0%, w/v, in diethyl ether). The reactions were performed in a standard GC vial fitted with a teflon-lined screw-cap lid. After reaction at 50°C in a GC oven for 48 h, the mixture was cooled and diluted with hexane (200 µl). Iodine was removed by shaking with 5% (w/v) aqueous $Na_2S_2O_3$ (100 µl). The organic layer was removed and the aqueous layer re-extracted with hexane : chloroform (4 : 1, v/v). The combined organic layers were

Table 1

Characteristic ion fragments of derivatized products formed by reaction of monounsaturated fatty acid methyl esters with dimethyldisulfide

Fatty acid methyl ester	Diagnostic ions (m/z)		
	M^+	Δ [a]	ω [b]
16:1ω7c	362	217	145
16:1ω5c	362	245	117
i17:1ω7c	376	217	159
17:1ω6c	nd [c]	245	131
18:1ω9c	390	217	173
18:1ω7c	390	245	145
20:1ω9c	418	245	173
20:1ω7c	nd	273	145

[a] Δ, Double bond position from carboxylic end of molecule.
[b] ω indicates double bond position from aliphatic end of molecule.
[c] nd, not detected.

concentrated under a stream of nitrogen prior to GC analysis. GC–MS analysis of the DMDS adducts showed major ions attributable to fragmentation between the two methylsulfide containing carbons of the original unsaturation position. Discrimination between *cis* and *trans* geometry of the double bond in the original monoenoic FAME is possible. The erythro isomer (originally the *trans* acid) elutes after the threo isomer (originally the *cis* acid). The different positional isomers of the same geometry were chromatographically separated under the GC conditions used in this study.

3.4. Fatty acid nomenclature

Fatty acids are designated as total number of carbon atoms: number of double bonds followed by the position of the double bond from the Δ (aliphatic) end of the molecule. The suffixes c and t indicate *cis* and *trans* geometry. The prefixes i and a refer to *iso* and *anteiso* branching, respectively. Other methyl-branching is indicated as position from the carboxylic acid (ω) end, i.e., 10 Methyl 16:0. Cyclopropane fatty acids are designated by the prefix cy.

3.5. Thymidine incorporation into DNA

Freshly collected 1-g sediment samples were added to disposable 15 ml centrifuge tubes with 30 μCi [^{3}H]thymidine (SA 79 Ci/mmol), 40 μl of 10 μM thymidine and 200 μl of a 0.3 mM solution of cycloheximide (eukaryotic inhibitor) [21] in filter-sterilized seawater (0.22 μm millipore) at −2°C. The sediment was incubated aerobically for 8 h at −2°C under low light (35 $\mu E \cdot m^{-2} \cdot s^{-1}$). These incubation conditions were found to be optimal by isotope dilution and time-course experiments [21]. In addition, 80% ethanol-killed controls were incubated in parallel with all samples to determine any unincorporated [^{3}H]thymidine carried through the DNA purification procedure. The purification for DNA was commenced by adding 6 ml of 80% ethanol and vortexing to stop incubation [22]. The samples were immediately centrifuged at 3000–5000 × g for 4 min. The ethanol was discarded, and 1 ml of 0.3 M NaOH containing 1% sodium dodecyl sulfate (SDS) and 10 mM thymidine was added to the sediment, which was then heated for 2 h at 100°C. The caps to the tubes were placed on loosely for the first 15 min to allow any remaining ethanol to escape. After cooling to room temperature samples were centrifuged (as before) and the supernatant collected into clean tubes. Distilled water (1 ml) was added to the sediment, the mixture was vortexed and centrifuged (as before), and the supernatants combined. The supernatants were then transferred to a dialysis membrane (6000 Da Cut-off, Spectrapor, Spectrum Medical Industries, Los Angeles, CA) and dialyzed against tap water for 8–12 h. The dialyzed liquid was transferred to centrifuge tubes and made up to a constant volume. Aliquots (1 ml) of the sample were transferred to scintillation vials and 10 ml of Aquasol II was added prior to counting on a Beckman LSC-100 using the standards ratio method.

3.6. Acetate incorporation into microbial lipids

Sediment samples (2 g wet wt.) were added immediately to 5 μCi sodium [^{14}C]acetate (SA 45–60 mCi/mmol) in 50-ml disposable centrifuge tubes. Aerobic incubation, 8 h at −2°C under low light (35 $\mu E \cdot m^{-2} \cdot s^{-1}$) [23], was stopped by the addition of methanol:chloroform (37.5 ml, 2:1, v/v). Following extraction for a minimum of 2 h, a further 25 ml of chloroform:water (1:1, v:v) was added. The resulting mixture was shaken vigorously and the two phases allowed to separate

overnight. The lower chloroform layer was collected, the solvent removed under a stream of nitrogen and an aliquot was counted after the addition of 10 ml Aquasol II. The remaining sample was separated using a silicic acid column (Unisil, 0.4 g, 100–200 mesh) into neutral, glyco- and phospholipid fractions [23]. The solvent was removed and the samples were prepared as above for counting.

3.7. Statistical analyses

The Florida State University Cyber 730 mainframe computer with SPSS programs was utilized for all analyses. The test of Tukey's Honestly Significant Difference (HSD, SPSS program) keeping the within-experiment, family-wise error rate set at $\alpha = 0.05$, was performed on fatty acid data.

4. RESULTS AND DISCUSSION

4.1. Phospholipid ester-linked fatty acid (PELFA) biomass estimates

Conversion of PELFA concentration data (Table 2) into number of cells per g of sediment can be performed using the following approximations [19]. Average bacteria the size of *Escherichia coli* contain 100 μmol PELFA/g (dry wt.) and 1 g of bacteria is equivalent to 5.9×10^{12} cells (dry wt.). Using these conversion factors, the following microbial cell concentrations (number of cells/g dry wt. of sediment) were determined: CA(1) 2.0×10^9, CA(2) 1.3×10^9, CE 4.5×10^9 and NH, an order of magnitude lower, 3.7×10^8. These microbial biomass estimations reveal a similar trend to those found by other investigators in McMurdo Sound [14,24,25]. West McMurdo Sound at New Harbor contained a lower sediment biomass than the East Sound sites. Cape Evans was found to contain the greatest biomass, followed by the Cape Armitage sites. New Harbor contained only 13% as much biomass when compared to the average total phospholipids of the East Sound sites.

Interestingly, the cell concentration range determined is equivalent to that in a subtropical Florida estuary [19] and is one and two orders of magnitude greater than the values determined for a deep-sea area subjected to abyssal storms, and a relatively undisturbed deep sea sediment (Venezuela) [6], respectively.

4.2. Community structure

PELFA profiles have been previously utilized to determine microbial community structure [1–3,18,26–28]. In this study determination of monounsaturated fatty acid double bond positions and geometry was performed (Table 1), as it is our belief that such data are essential for correct interpretation of increasingly complex ecological and taxonomic data sets.

4.2.1. Diatoms

The acids: 16 : 1ω7c, 14 : 0, 16 : 0, 20 : 5ω3 and 20 : 4ω6 (Table 2), which are typically major components in diatom lipids [28–32], were present in high proportions in the PELFA of all Antarctic sediments analysed. Although these components may also be input from other sources, their high relative and absolute abundances are consistent with a major diatom contribution. C_{16} polyunsaturated fatty acids (PUFA) have been reported as relatively major components in the *Bacillariophyceae* (e.g., [30]). The low proportion of these components in this study is thus interesting. Analysis of an Antarctic sea-ice diatom showed that the relative abundance of C_{16} PUFA was significantly greater (5 ×) in glycolipids than in phospholipids [32]. The low proportions of C_{16} PUFA in the isolated sedimentary phospholipid could therefore be due to differing proportions of these components within the diatom lipid classes. Alternatively, diatoms other than those containing high proportions of these C_{16} components may be the dominant species present. Further supporting evidence (White et al., unpublished data; [33]) for the observed diatom contribution are: (i) the high proportions of sedimentary neutral lipid (mainly triglyceride) and glycolipid fatty acids as observed for diatom lipids [32]; (ii) sterol and carotenoid abundances and profiles; and (iii) microscopic examination revealed that the actively photosynthesizing diatom *Trachyneis aspera* was abundant in the surface sediments of the East Sound

Table 2

Phospholipid fatty acid profiles of four sediments from McMurdo Sound, Antarctica

Fatty acid [a]	RT [b]	% Composition [c]			
		CA(I) [d]	CA(2)	CE	NH
i14:0	16.48	0.4	tr [f]	0.4	tr
14:0	17.12	1.5	1.1	1.6	1.2
i15:1ω5c [e]	17.88	0.3	tr	tr	tr
i15:0	18.31	1.1	1.0	0.8	0.8
a15:0	18.46	1.6	1.6	2.0	1.1
15:0	19.01	0.5	0.5	0.7	0.5
16:4ω1 [e]	19.98	0.3	tr	tr	tr
16:3ω4 [e]	20.16	0.6	0.6	1.6	0.8
i16:0	20.30	0.7	0.8	0.5	0.6
16:1ω9c	20.43	3.6	3.4	1.8	3.2
16:1ω7c	20.55	22.4	19.2	24.3	17.8
16:1ω7t	20.62	0.4	0.7	0.4	1.0
16:1ω5c	20.72	0.9	2.2	1.5	1.1
16:1ω13t	20.85	1.0	0.6	0.7	0.7
16:0	21.07	11.7	10.7	12.6	13.2
i17:1ω7c	21.76	0.4	0.4	0.3	0.3
10Me16:0	21.95	0.7	1.2	tr	0.6
i17:0	22.36	0.6	0.7	0.4	0.7
a17:0 [g]	22.52	1.8	1.6	1.2	1.2
17:1ω6c	22.69	0.7	0.8	1.5	0.6
cy17:0	22.74	tr	0.3	tr	tr
17:0	23.11	0.4	0.5	0.7	0.5
18:3ω6 [e]	24.05	tr	tr	0.4	0.6
18:4ω3 [e]	24.11	1.2	1.7	0.7	0.8
18:2ω6	24.38	0.4	0.5	0.5	0.6
18:3ω3	24.46	tr	tr	0.3	tr
18:1ω9c	24.62	5.5	5.4	3.7	4.6
18:1ω7c	24.76	16.3	16.7	16.8	7.5
18:1ω7t	24.82	tr	0.4	0.4	tr
18:1ω5c	24.93	0.3	0.4	0.4	0.4
18:0	25.24	1.0	1.5	1.5	1.9
cy19:0	26.99	0.3	0.5	tr	0.4
20:4ω6	27.72	1.7	3.6	1.1	6.7
20:5ω3	27.79	13.7	10.0	13.2	15.3
20:3ω6 [e]	28.25	0.5	0.4	0.3	0.5
20:4ω3 [e]	28.31	tr	0.3	tr	tr
20:2ω6 [e]	28.58	tr	tr	tr	0.8
i20:0	28.62	0.3	tr	tr	tr
20:1ω11c [e]	28.66	0.4	tr	1.2	0.7
20:1ω9c	28.76	0.5	1.0	tr	tr
20:1ω7c	28.94	tr	0.6	0.7	1.1
20:0	29.43	0.3	tr	tr	tr
21:0	31.47	3.1	0.4	0.3	0.5
22:6ω3	31.57	0.4	2.9	3.2	6.3
22:4ω6	31.79	tr	0.3	0.6	1.2
22:5ω3	31.87	tr	0.5	tr	tr
22:3ω6 [e]	32.29	tr	tr	tr	0.3
22:4ω3 [e]	32.37	tr	tr	tr	tr
22:1ω11c [e]	32.80	tr	tr	tr	0.3
22:1ω9c	32.88	tr	tr	tr	0.3
22:0	33.42	0.7	2.2	tr	1.4
23:0	35.41	tr	tr	tr	tr
24:1ω9c	36.75	tr	tr	tr	tr
24:0	37.29	tr	tr	tr	tr
25:0	39.08	tr	tr	tr	tr
26:1ω9c	40.38	tr	tr	tr	tr
26:0	40.86	tr	tr	tr	tr
Total tr [h]		1.4	2.8	1.5	1.9
Total FAME [i]		34.6	22.8	75.7	6.2

[a] Fatty acid identifications based on GC retention data, GC–MS confirmation and analysis of dimethyldisulfide adducts of monounsaturated components (Table 1), unless otherwise specified.

[b] RT, retention time in min, separation on a Hewlett Packard 50 m×0.25 mm non-polar CMS fused silica capillary column.

[c] Expressed as % total fatty acids.

[d] CA(1), Cape Armitage 14.5 m; CA(2), Cape Armitage 20 m; CE, Cape Evans 20 m; NH, New Harbor 30.5 m.

[e] Identification based on GC retention data alone.

[f] tr, Trace amounts of fatty acids at or below selected cutoff of 0.2%.

[g] Includes 17:1ω8c.

[h] Total of all trace components of 0.2% or less.

[i] Total amount of FAME in nmol/g dry wt.

([34]; A. Palmisano, personal communication).

The presence of a number of other unsaturated components, 16:3ω4, 16:1ω13t, 22:5ω3, 22:4ω6, and 22:6ω3 (Table 2), can also be rationalized as due, at least in part, to a direct diatom contribution [26]. The fatty acid composition of the sea ice diatom *Nitzschia cylindrus*, a major component of the sea ice community, has recently been analyzed in this laboratory. Saturated and relatively novel monounsaturated C_{24} and C_{26} fatty acids accounted for 20% of the PELFA of this sea ice diatom. The low relative abundance of these components in the Antarctic benthic marine sediments analyzed here (Table 2) suggest a small contribution from this organism to the viable benthic community. Sediment trap experiments and analysis of the benthic diatom community are planned to gain further insight into the abundance of specific organisms in the benthic environment.

Comparison of fatty acid profiles obtained for

the 4 sites reveals a number of trends with respect to the diatom markers discussed above. High ratios of 16:1ω7c/16:0 have been previously reported in a study which calculated the contribution of diatoms to benthic marine sediments [26,35]. Cape Evans sediments contained the greatest relative amount of 16:1ω7c, followed by the 2 Cape Armitage sites (Table 3). The ratio of 16:1ωc/16:0 (Table 4), also indicates a somewhat greater diatom biomass at the East Sound sites. Results of Tukey's HSD test (Table 3) performed on the sedimentary fatty acid profiles confirmed this preliminary observation. The Cape Evans site generally contained the highest proportion of the diatom markers discussed above.

Table 3

Phospholipid ester-linked fatty acids from sediments at four McMurdo Sound, Antarctica sites

Tukey's honestly significant difference test, with family group error set at $\alpha = 0.05$.

Fatty acid	Lowest [a]			Highest
i14:0, 18:1ω7c, 20:5ω3, [b]				
22:6ω3	NH	CA(2) [c]	CA(1)	CE
14:0	NH	CA(2)	CA(1)	CE
i15:1, 16:3ω4	NH	CA(2)	CA(1)	CE
i15:0, i16:0, 18:1ω9c	NH	CA(2)	CA(1)	CE
al5:0, 16:4ω3, 17:0, 18:3ω3				
22:4ω6	NH	CA(2)	CA(1)	CE
16:1ω9c	NH	CA(2)	CA(1)	CE
16:1ω7c, 20:4ω6c	NH	CA(1)	CA(2)	CE
16:1ω7t, 16:1ω13t, i17:1ω7c				
17:1ω6c	NH	CA(2)	CA(1)	CE
16:1ω5c	NH	CA(1)	CA(1)	CE
16:0	NH	CA(2)	CA(1)	CE
10Me16:0	NH	CE	CA(1)	CA(2)
i17:0	NH	CA(2)	CA(1)	CE
a17:0	NH	CA(2)	CA(1)	CE
cy17:0	NH	CA(1)	CE	CA(2)
18:4ω3	NH	CA(2)	CA(1)	CE
18:1ω5c, 18:0	NH	CA(1)	CA(2)	CE
cy19:0	NH	CE	CA(1)	CA(2)
22:5ω3	CE	NH	CA(1)	CA(2)

[a] Sites are ordered from lowest to highest according to nmol/g dry wt. values, and sites connected by a common line are not significantly different.

[b] Grouped fatty acids show the same significance using the test.

[c] CA(1), Cape Armitage 14.5 m; CA(2), Cape Armitage 20 m; CE, Cape Evans 20 m; NH, New Harbor 30.5 m.

Table 4

Ratios of selected phospholipid ester-linked fatty acid pairs for four Antarctic benthic sediments

Fatty acid	CA(1) [a]	CA(2)	CE	NH
i+a 15:0/16:0	0.22	0.24	0.21	0.15
i+a 15:0/16:1ω7c	0.12	0.13	0.11	0.11
i+a 15:0/20:5ω3	0.20	0.26	0.20	0.13
i+a 15:0/20:4ω6	1.58	1.86	1.98	1.43
10Me16:0/16:0	0.06	0.11	0.01	0.04
cy17:0/16:0	0.01	0.03	0.01	0.01
16:1ω7c/16:0	1.91	1.80	1.93	1.35
20:4ω6/16:0	0.14	0.34	0.09	0.51
20:5ω3/16:0	1.17	0.93	1.05	1.16
20:4ω6/16:1ω7c	0.07	0.19	0.05	0.37
18:1ω7c/16:1ω7c	0.73	0.87	0.70	0.42
18:1ω7c/6:0	1.39	1.56	1.34	0.57

[a] CA(1), Cape Armitage 14.5 m; CA(2), Cape Armitage 20 m; CE, Cape Evans 20 m; NH, New Harbor 30.5 M.

4.2.2. Bacteria

It is recognized that certain fatty acids are specific to bacteria and that different groups of bacteria have different fatty acid compositions [1–3,18,28]. Although differences observed for complex environmental samples have been rationalized using a bacterial signature lipid approach, it is clearly of more use to obtain information on the proportions of metabolic groups within an environment. At the present time an increasing proportion of complex environmental samples can be rationalized in terms of their fatty acid profiles into metabolic subgroups when detailed analysis, including determination of double bond configuration and position, is performed.

A number of similarities and differences for specific bacterial PELFA between the 4 Antarctic sediments sites are recognized. The saturated branched and odd-carbon fatty acids, i14:0, i15:0, a15:0, 15:0, i17:0, a17:0 and 17:0, are present in similar relative proportions at the 4 sites (Table 2). Ratios of i+a15:0 and 18:1ω7c to 16:0 showed similar trends to those of the microbial biomass and activities, with the East Sound sites being greater than the West in bacterial components by 32 and 60%, respectively (Table 4). In addition to the overall bacterial biomasses of the sites, higher ratios of the bacterial marker lipids i+a 15:0 to a common microeukaryote marker (20:4ω6), indicated slightly higher proportions of

bacteria at the East Sound sites (Table 4). A similar trend was also observed for the ratios of i + a15 : 0 to the PUFA 20 : 5ω3 (Table 4).

The Cape Armitage site at 20 m, (CA(2)) showed a significantly increased amount of marker lipids from acetate-utilizing sulfate-reducing bacteria (SRB) (Table 2). Acetate-utilizing SRB belonging to the genus *Desulfobacter* have been isolated from CA sediments in concurrent studies being undertaken in our laboratory (Dowling and White, unpublished data). The fatty acids 10Me16 : 0 and cyclopropyl 17 : 0 are major components of the sulfate-reducing genus *Desulfobacter* in marine systems [36]. These two components were also detected as major acids in all positively identified *Desulfobacter* [37], thus supporting their use as markers for this genus. The ratios of 10Me16 : 0 and cy17 : 0 to 16 : 0 were greater by 68 and 76% respectively, from the average of the other 2 East Sound sites and the West Sound site (Table 4). The acid 10Me16 : 0 is known to be a minor component of terrestrial actinomycetes [38]; however, other 10 methyl fatty acids are also in equally high abundance in actinomycetes. Thus, the presence of only 10Me16 : 0 at the CA(2) site is consistent with the assumption that a higher proportion and biomass of *Desulfobacter* occurs at this site.

The branched chain odd carbon numbered fatty acid i17 : 1w7c has been detected as a major fatty acid in members of the genus *Desulfovibrio* [36,39,40], and also more recently in several *Flexibacter* [41]. The second group of organisms contained, in addition to i17 : 1ω7c, high proportions of i17 : 1ω5c. This latter acid was not present in the Antarctic sediments analyzed by GC–MS (following derivatization of the component monounsaturated fatty acids). The presence of i17 : 1ω7c and i15 : 1ω5c in all the samples is, at the present time, taken to be indicative of the genus *Desulfovibrio*. In contrast to the varying relative proportions of *Desulfobacter* markers, relatively similar amounts of i17 : 1ω7c and i15 : 1ω5c were present at all 4 sites (Table 2). From mean PELFA compositional data obtained for pure cultures of *Desulfobacter* [37] and *Desulfovibrio* [40], it can be estimated that these bacteria account for 5–10% and 1–2%, respectively, of the total microbial biomass in the Antarctic benthic sediments of McMurdo Sound. Bacterial sulphate reduction within reduced microniches of oxidized marine sediments has been previously demonstrated using a radiotracer technique [42]. The occurrence of low proportions of SRB markers within the upper aerobic layer of Antarctic sediments is in agreement with those previous findings.

Vaccenic acid (18 : 1ω7c) has been widely proposed as an indicator of bacterial input in sediments [26,29,43]. An increasing number of organisms, including diatoms, have also been found to contain this fatty acid [29,32,44]. Production of 18 : 1ω7c via chain elongation of 16 : 1ω7c has been proposed [26]. Diatoms, although they may contain this fatty acid, typically have low proportions of C_{18} fatty acids [27,29–32,51]. The high relative abundance of 18 : 1ω7c (Tables 2 and 4) at all 4 sediment sites is thus interpreted as being due to the presence of high proportions of the 18 : 1ω7 containing bacterial chemotype, in agreement with PELFA ratio data (Table 4).

The *trans* acids 16 : 1ω7t and 18 : 1ω7t were detected as minor components in sediments from all sites (Table 2), further extending the range of environments from which *trans* acids have been isolated. It has been proposed that *trans* acids originate from microbial and/or abiological degradation of *cis* mono- and polyunsaturated fatty acids [46–48], and from direct bacterial input [43,48]. It is our belief that the presence of *trans* acids in the membrane originating PELFA of Antarctic benthic sediments may be due to direct microbial input.

The ratio of *trans* to *cis* components for both 18 : 1ω7 and 16 : 1ω7 may be utilized as a starvation or stress lipid index, which may aid in determining the nutritional status of bacteria in natural aquatic environments [49]. Comparison of starved cultures of *Vibrio cholerae* revealed large increases in the *trans*/*cis* ratio with time [49]. The ratios observed for the PELFA of the Antarctic sediment studied were similar to values obtained for healthy, unstarved cells. These data suggest that PELFA ratios may be used to monitor the physiological state of organisms in pure cultures [49], and in the natural environment (unpublished data; and G. Odham, personal communication).

4.3. Incorporation of labeled substrates

Metabolic activities indicated a similar pattern to that of the cell concentrations calculated from PELFA content of the sediments [33]. New Harbor sediment showed the lowest incorporation of [^{3}H]thymidine into bacterial DNA and sodium [^{14}C]acetate into membrane lipids (Table 5). The metabolic activity of the East Sound sites were on the average 45 and 73% higher in their incorporation of thymidine and sodium acetate, respectively. The increase in lipid synthesis suggested by the data in Table 5 assumes that the acetate pool size at these sites parallels the thymidine pool measured by isotope dilution [21]. Unfortunately, the acetate pool size at each site was not measured. Measurements of [^{3}H]thymidine incorporation gave rates of cell division 4–5 orders of magnitude less than those of a North Australian seagrass bed sediment [22]. The average rate for the 4 sites was found to be 7.6×10^4 bacterial cell divisions per h per g sediment. It is known that acetate-utilizing SRB do not incorporate [^{3}H]thymidine [21]. Although this group of organisms was present in the Antarctic sediments studied (section 4.2.2), their relative low abundance (approx. 5–10%, by PELFA calculations), should not cause a significant underestimation of productivity using the [^{3}H]thymidine procedure. Incorporation of sodium[^{14}C]acetate gave rates of membrane lipid synthesis averaging 39000 dpm per h per g sediment.

4.4. Factors affecting biomass and activity

The greatest influence upon the biomass and activity of the benthic microorganisms appears to be the current flow. The predominant flow enters the Sound under the annual ice on the East side and travels South along Ross Island and under the Ross Ice Shelf (permanent ice). The returning current comes from under the shelf and travels along the West Sound [14,25]. This water has been found to be low in bacterioplankton biomass, based on chlorophyll *a* data, and metabolic activity measured using turnover rates of dissolved ATP D-glycose and L-leucine [24,25,50]. The benthic macroinvertebrates are also found in reduced number and diversity from the East Sound sites [14,51].

In addition to the current regime of the Sound, the West Sound retains its sea ice over most of the area for the entire year [14,52]. This results in a severe light reduction to the benthos. The snow and sea ice has a very high light extinction coefficient and was found to reduce the under-ice irradiance to 10 to $< 0.01\%$ of surface irradiance through the average 2 m snow and ice thickness [52]. The downwelling irradiance through the snow cover, ice and water column for a Cape Armitage site at 20 m depth was found to be below 0.6 $\mu E \cdot m^{-2} \cdot s^{-1}$, but enough to maintain a sufficient diatom community [34]. There is apparently a smaller benthic diatom population at the New Harbor site investigated, based on the PELFA

Table 5

Benthic microbial metabolic activity of four sediment sites in McMurdo Sound, Antarctica

	NH	CA(2)	CA(1)	CE
[^{3}H]Thymidine (bacterial DNA) [b]	4720 [a] (2934) (0.006) [c]	5830 (4498) (0.009)	7190 (2327) (0.01)	10300 (4271) (0.02)
	NH	CA(1)	CE	CA(2)
Sodium[^{14}C]acetate (membrane lipid)	12900 (7530) (0.1)	33500 (33483) (0.3)	39300 (15840) (0.4)	70500 (18551) (0.6)

[a] dpm/h/g wet wt., numbers represent the mean of 5 samples with the mean of 3 controls subtracted (SD).
[b] Thymidine incorporations do not include sulfate-reducing bacteria.
[c] Numbers in parentheses represent label incorporated as % dpm added (dpm incorporated/dpm in inoculation).

data of this study, however, input to the benthos may also be derived from the sea-ice communities which are equal or greater than those of the East Sound [15].

The information on sediment lipid composition and metabolic activity presented in this report verifies earlier productivity measurement from this area, and expands the existing knowledge of microbial community structure within these marine sediments. Further investigation into sea-ice microalgal input and its fate in the food chain is presently underway in this laboratory.

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