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Methanogenesis and microbial lipid synthesis in anoxic salt marsh sediments

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Abstract. In anoxic salt marsh sediments of Sapelo Island, GA, USA, the vertical distribution of CH_4 production was measured in the upper 20 cm of surface sediments in ten locations. In one section of high marsh sediments, the concentration and oxidation of acetate in sediment porewaters and the rate and amount of ^{14}C acetate and $^{14}CO_2$ incorporation into cellular lipids of the microbial population were investigated. CH_4 production rates ranged from < 1 to 493 nM CH_4 gram sediment^{-1} day^{-1} from intact subcores incubated under nitrogen. Replacement with H_2 stimulated the rate of methane release up to nine fold relative to N_2 incubations. Rates of lipid synthesis from CO_2 averaged 39.2 × 10^{-2} nanomoles lipid carbon cm^3 sediment^{-1} hr^{-1}, suggesting that CO_2 may be an important carbon precursor for microbial membrane synthesis in marsh sediments under anoxic conditions. Qualitative measurements of lipid synthesis rates from acetate were found to average 8.7 × 10^{-2} nanomoles. Phospholipids were the dominant lipids synthesized by both substrates in sediment cores, accounting for an average of 76.6% of all lipid radioactivity. Small amounts of ether lipids indicative of methanogenic bacteria were observed in cores incubated for 7 days, with similar rates of synthesis for both CO_2 and acetate. The low rate of ether lipid synthesis suggests that either methanogen lipid biosynthesis is very slow or that methanogens represent a small component of total microbial lipid synthesis in anoxic sediments.

Introduction

In marine sediments, the complete mineralization of organic matter requires the integration of several bacterial groups (Fenchel & Blackburn 1979). In anaerobic environments several intermediates play a key role in the process of organic carbon mineralization with acetate, CO_2 and H_2 appearing as the predominant compounds (e.g. Ward & Winfrey 1985). These intermediates are produced as end products of polymeric organic matter decomposition by the fermentative and obligate proton reducing bacteria, and are subsequently consumed by the methanogenic and sulfate reducing bacteria in the terminal

stage of organic carbon degradation to CH_4 or CO_2. Where sulfate is not limiting, sulfate reduction is thought to predominate over methanogenesis (Martens & Berner 1974; Winfrey & Ward 1982) reflecting the higher affinity (lower Km) of sulfate reducers (verses methanogenic bacteria) for both hydrogen and acetate (Kristjansson et al. 1982; Schonheit et al. 1982). The two processes are not mutually exclusive, however, and significant rates of methanogenesis have been observed in sediments undergoing vigorous sulfate reduction (Oremland et al. 1982; Senior et al. 1982) which may reflect the utilization of "noncompetitive" substrates such as methylated amines and methanol by the methanogens.

The salt marsh sediments from Sapelo Island, GA, USA, were the subject of the present study. Organic matter in these sediments is primarily from the marsh grass *Spartina alterniflora* Loisel (Pomeroy & Wiegert 1981), and the high rate of organic matter input results in permanently anoxic conditions within a centimeter of the sediment surface (Howes et al. 1981). Several anaerobic processes have been measured in these sediments including sulfate reduction (Skyring et al. 1979; Howarth & Giblin 1983), methane evolution (King & Wiebe 1978, 1980; Jones & Paynter 1980) and the metabolism of glucose (Christian & Wiebe 1978). In this study two additional facets of anaerobic microbial metabolism were investigated; the influence of H_2 on the vertical distribution of sedimentary methanogenesis in intact sediment subcores and the importance of acetate and CO_2 as precursors for microbial membrane lipid synthesis. At least two chemical groups of membrane lipid are synthesized in these sediments. The membrane lipids of most bacteria and other sediment organisms contain fatty acids linked to glycerol through an ester bond. In contrast, the lipids of the methanogenic bacteria and other *Archaebacteria* contain isoprenoid alkanes in ether linkage to glycerol as the predominant feature (Tornabene & Langworthy 1978; Kushwaha et al. 1981). Taking advantage of these functionally similar but chemically distinct types of lipids, the goals of the present study were to examine:

- the relative importance of acetate and CO_2 as precursors for lipid synthesis in sediments where active methanogens were present, and
- the influence of H_2 on the vertical distribution of sediment methanogenesis.

Materials and methods

Sediment sampling and organic matter measurement

All experiments were carried out in April and May, 1984. At each site, the upper 25 cm of sediment was sampled at low tide in areas between stands of

vegetation with pvc cores (6 cm i.d.) which were slowly inserted by hand to minimize any sediment compaction. Cores were capped with butyl rubber stoppers and returned to the laboratory for processing within one hour. All subsequent measurements were made in the upper 20 cm of surface sediment which was divided into 5 cm sections (0–5, 5–10, 10–15, and 15–20 cm) using cut-off syringes inserted into each core. After removal of small amounts of visible roots and rhizomes, sediment organic matter in each section was determined as percent by weight. Triplicate samples were dried at 90 °C to constant weight and percent organic matter determined as loss by combustion at 450 °C for two hours, followed by reequilibration at drying temperatures.

Measurement of below surface methanogenesis

The six sites in which methane production was examined at 5 cm intervals are illustrated in Fig. 1. The sediments comprised 4 high marsh sites (sites 1–4) which are flooded only during spring phase high tides, and two transects at sites A and B. Each transect included a sample from near the tidal creekbanks, intermediate sediments approximately equal distance between high and low tidal levels, and high marsh sites above the mean high tide mark. At each site and depth, duplicate syringes containing the 5 cm sediment subcore were extruded into 15 ml Vacutainers (Terumo Medical Corp., Elkton, MD) while being continually flushed with nitrogen. The Vacutainer stoppers were immediately replaced and the subcores incubated at the average sediment temperature (20 $\pm$ 1°C) for 24 h. The influence of hydrogen on methanogenesis was examined in duplicate samples of identically prepared subcores in which the entire headspace was replaced with H_2 using needles inserted through the stopper. Control sediments consisted of subcores injected with 1 ml of 37% formaldehyde. Methane produced was quantified using a Varian 3700 gas chromatograph equipped with a Poropack Q (80–100 mesh) column and flame ionization detection. The column was operated at 160 °C with nitrogen as the carrier gas at a flow rate of 30 ml min^{-1}. Following CH_4 measurement, the stoppers were removed and samples dried (90 °C to constant weight). Wet weight/dry weight ratios (avg. porosity 81.6%) were used to determine methane dissolved in pore waters (salinity 30‰), and to calculate methane production rates.

Porewater measurements

Sediment porewater from each 5 cm sediment section of the Shell Hammock high marsh (site 2) was obtained by centrifugation under nitrogen at 12 000 x G for 20 min. Porewater samples were removed by pipette, split

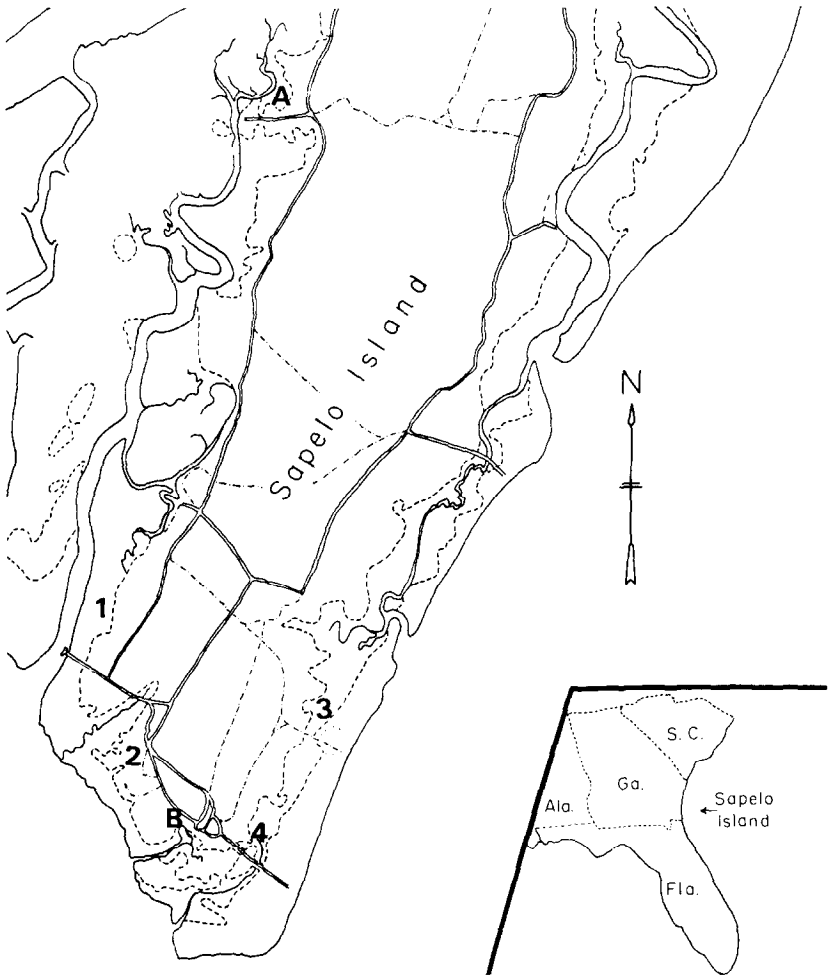

Fig. 1. Location of the six sampling sites surrounding Sapelo Island. Site 1 – Airport marsh, 2 – Shell hammock, 3 – Old Beach Road and 4 – Deans Creek. Transects A and B include three sites consisting of creekbank, intermediate and high marsh sites. Site designations refer to CH_4 production rates in Figs. 2 and 3.

into subsamples and frozen until analysis. One percent zinc acetate (v/v) was added to subsamples for porewater sulfate analyses immediately after centrifugation to prevent overestimates due to the oxidation of sulfides. Total porewater CO_2 was quantified by IR analysis of acidified samples after 10 fold dilution (Fallon & Brock 1980) while SO_4 was quantified in similarly diluted samples by $BaSO_4$ precipitation (Roy & Trudinger 1970). Salinity

was determined by conductance of 10 fold diluted samples at 25 °C with creek water of known salinity as the calibration standard. Porewater acetate was determined using the method of Christensen & Blackburn (1982) with slight modifications. Briefly, 500 µl porewater samples were acidified with 100 µl of 0.5 M H_3PO_4 to pH 1 & vacuum distilled to separate volatile acids from non-volatile components. The recovery of acetate from porewater "spiked" with either ^{14}C acetate or known concentractions of unlabeled acetate averaged 84.4% (± 6.7%) from distilled samples. Acetate concentrations in distilled porewaters were then quantified using a Hewlett Packard 5840A integrating gas chromatograph with flame ionization detection. A glass column with a support of Graphpac GC (60–80 mesh) and liquid phase of 0.3% Carbowax 20 M + 0.1% H_3PO_4 (Alltech Inc.) separated acetate from other volatile acids and was operated at 120 °C with detector maintained at 200 °C. Carrier gas (N_2) was supplied at 25 ml min^{-1}. All samples were injected directly on column using a positive displacement syringe. All porewater samples were measured in triplicate, corrected for recovery efficiency, and concentrations calculated from integrated peak areas compared to standards injected before and after distilled samples. Whenever peak tailing or broadening occurred (ca. every 20 injections), the column was reconditioned by injections of 1% formic acid in distilled water.

Lipid synthesis in sediments

The incorporation of CO_2 and acetate into lipids of the endogenous microbial population was conducted within the 10–15 cm sediment layer of Shell Hammock sediments. This site and depth was chosen for its high CH_4 production, and the absence of rooted vegetation below 10 cm. After the upper sediments had been extruded and removed, three ml cut-off syringes were inserted into the 10–15 cm sediment layer. Surrounding sediment was removed and stoppers immediately placed over the ends, sealing a section of sediment within the syringe. Either 10 µl (7.88 ICi–0.138 µmoles) of 1-^{14}C acetate (Research Products Int.) or 20.0 µl (20.0 µCi-1.00 µmole) of ^{14}C bicarbonate (New England Nuclear) was injected through the stoppers along the center of each section to distribute the substrate evenly along the center of each subcore while minimizing disturbance to the intact sediment. Based on porewater measurements, concentrations of acetate and CO_2 increased in porewater by 37.4% and 2.3%, respectively. Sediment cores injected with substrates in 10% (v/v) formaldehyde were used as killed controls. Acetate injections were incubated in the dark at 20 °C for 20 min, 6 h, 12 h, 24 h, and 7 days. Cores injected with ^{14}C bicarbonate were incubated in the dark for longer periods of time (1 h, 1, 2, 3, and 7 days) to

account for the lower specific activity (0.412 nCi nM^{-1} at time 0) compared to acetate (21.4 nCi nM^{-1}). Although the high concentrations of sulfide present poised the sediments at low redox potentials (< -200 mV), the sealed cores were also kept in saturated brine solutions to reduce oxygen diffusion (Howarth & Giblin 1983).

At each time interval, duplicate subcores were extruded into 25 ml extraction vials containing a mixture of methanol-distilled water (1:1) and quickly stoppered. Samples were slurried on a vortex mixer, and CO_2 and CH_4 present in headspace gases flushed and quantified as described elsewhere (Harvey et al. 1986). Following the exchange of headspace gases, lipids were extracted using the method of Bligh & Dyer (1959) modified for the use of capped tubes. The lipid containing solvent layer was evaporated to dryness, redissolved in chloroform-methanol (1:1), and the amount of radioactivity incorporated into various lipid classes quantified by a combination of thin-layer chromatography (tlc), and liquid scintillation counting. To distinguish the synthesis of ester lipids in the majority of the microbial population from ether lipids typical of methanogenic bacteria, one half of the total extract was taken to dryness and saponified in 0.1 N methanolic KOH for 3 h at 40 °C to hydrolyze all ester linked moieties. Similar treatment of lipids from *Methanococcus voltae* strain ps and *Methanobacterium thermoautotrophicum* showed no evidence of ether lipid hydrolysis. In contrast, treatment of phospholipids and other ester linked lipids from a number of sources yielded greater than 95% hydrolysis. After the hydrolysis procedure, the neutral and polar lipid fractions were partitioned into hexane and the extracts pooled for separation by tlc. Neutral lipids were examined in the solvent system of petroleum ether/diethyl ether/acetic acid (85:15:1) and polar lipids were separated in the solvent system of chloroform/petroleum ether/methanol/acetic acid (50:30:16:10). Lipid class identification was made by comparison of Rf values with authentic phospholipid and ether lipid standards and reactivity with specific spray reagents (0.5% α-napthol/H_2SO_4 for glycolipids; periodate-Schiff for vicinal-OH groups and MoO_3/H_2SO_4 for phosphatides (Kates 1972)). Ether lipids were thus defined as those lipids showing similar Rf values in the polar solvent system both before and after the alkaline hydrolysis treatment. After class identification, lipid spots were scraped and incorporated radioactivity determined in 10 ml of scintillation cocktail using a Beckman LS 9000 liquid scintillation counter with external quench correction.

Acetate oxidation & binding

The oxidation of acetate and its binding to sediments during lipid synthesis was followed in an additional set of sample cores from high marsh sediments

of site 2. Sediment subcores were prepared and injected with 1-^{14}C acetate similarly to lipid incorporation experiments. At defined time intervals, cores were extruded into centrifuge tubes and porewater separated by centrifugation in sealed tubes. Any $^{14}CO_2$ produced from the oxidation of 1-^{14}C acetate was first removed from porewater samples by acidification to pH 2 with 5 N HCL and sparging of headspace gases with nitrogen (100 ml min^{-1} for 10 min). Exiting air was passed through a series of scintillation vials containing a CO_2 trapping fluor and $^{14}CO_2$ then quantified by scintillation counting (Harvey et al. 1986). Remaining radioactivity in the acidified porewater (as acetate or its non-volatile metabolites) was then measured by the addition of 10 ml of scintillation cocktail and counting. Using this technique, a small amount of acetate (3.2% $\pm$ 0.7%) is volatilized from the porewater sample and transferred to the CO_2 counting cocktail. Concentrations of $^{14}CO_2$ and ^{14}C-acetate (or its metabolites) present in sediment porewaters were therefore corrected for the volatilization of acetate by using the amount of free acetate found in porewaters at each time point.

Results

Methane production

The production of CH_4 and percent organic matter within the ten salt marsh sediments examined are shown in Figs. 2, 3. Methane production in control sediments injected with formaldehyde were all less than 10% of experimental rates. Highest CH_4 production rates occurred with the replacement of hydrogen as the headspece gas to subcores of high marsh sediments, the increase most evident in sediments from Airport marsh (site 1) and Shell Hammock (site 2). In these two areas, methanogenesis increased as much as nine fold over N_2 incubations. In sediment transects, increasing CH_4 production was apparent with increasing distance from tidal creeks. In both transects H_2 also stimulated CH_4 production, with the greatest increases occurring at high marsh sites. Although percent organic matter was generally higher in sediments with substantial CH_4 production, no significant correlation ($p = 0.72$) with organic matter was evident.

Porewater measurements

Sulfate, acetate and CO_2 concentrations within the upper 20 cm of high marsh sediments (site 2 – Fig. 2) are shown in Fig. 4. Acetate and sulfate showed the highest concentrations in the uppermost (0–5 cm) sediment section. Porewater sulfate concentrations showed an initial decrease in the

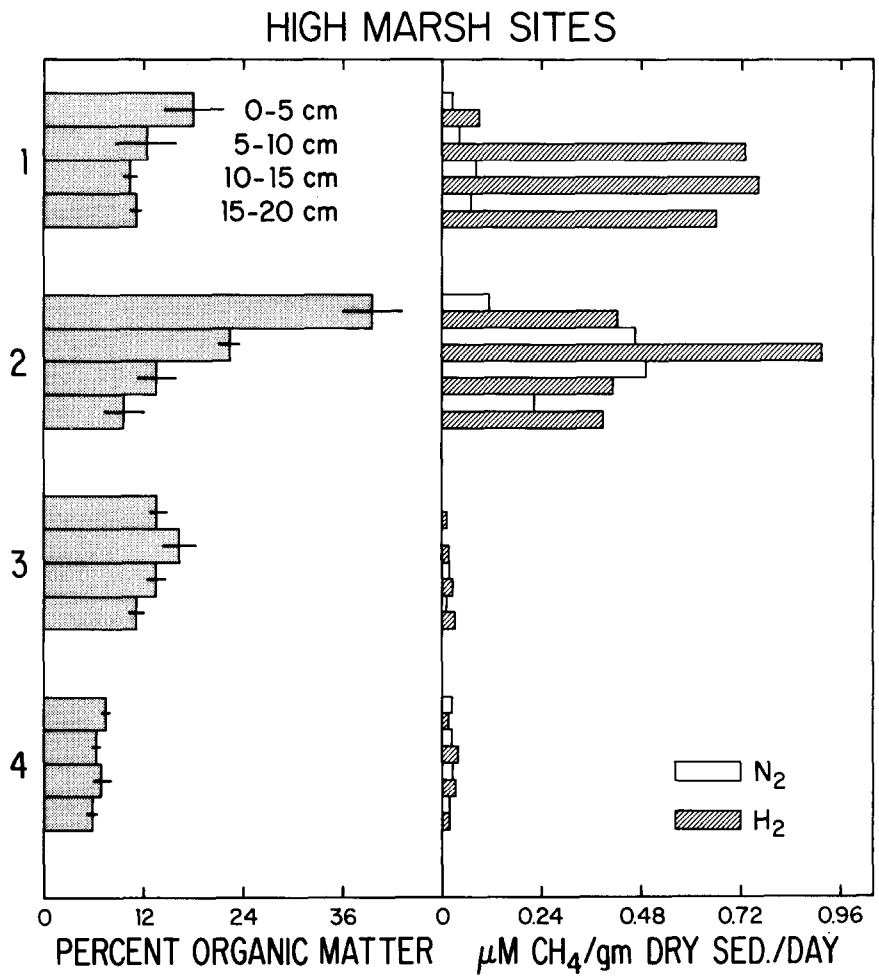

Fig. 2. The vertical distribution of methane production and percent organic matter within the upper 20 cm of four high marsh sediments. Error bars represent ±2 standard errors (SE) of the mean (n = 3).

upper 5 cm sediment section to less than 40% of concentrations in the adjacent creek, but declined only slightly thereafter. At increasing depths, acetate concentrations displayed a more gradual decrease with overall concentrations reduced over 50%. In contrast, dissolved CO_2 concentrations exhibited very little variation and remained at least 3 orders of magnitude greater than acetate at all sediment depths. Concentrations of other volatile fatty acids over all depths averaged 62 μM for isobutyrate and 213 μM for butyrate with little vertical variation observed in their distribution (data not shown). No significant changes were observed in porewater salinity which averaged 30 ± 2‰ at all depths.

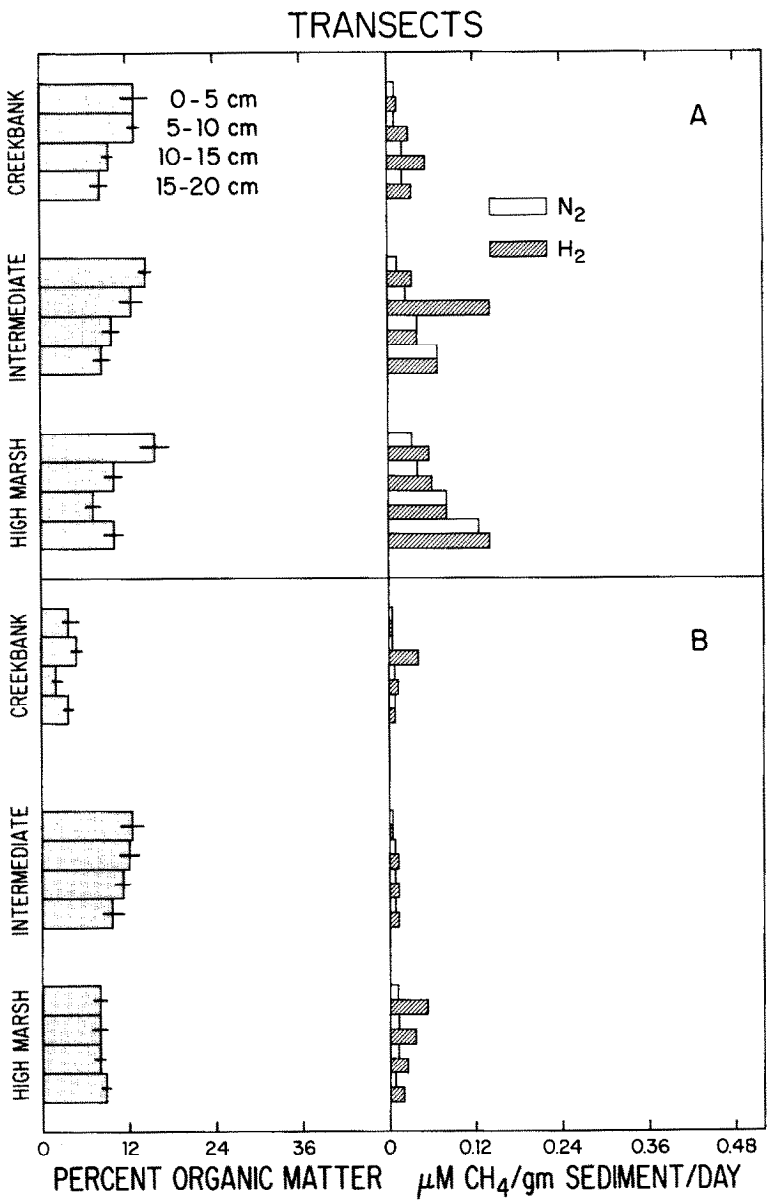

Fig. 3. The vertical distribution of methane production and percent organic matter within the upper 20 cm of the two sediment transects spanning tidal creekbanks to above mean high tide. Error bars equal ± 2 SE of the mean (n = 3).

Lipid synthesis in high marsh sediments

The amount and rates of ^{14}C-acetate and $^{14}CO_2$ incorporation into the lipids of the sediment community are shown in Table 1. The rates of lipid incor-

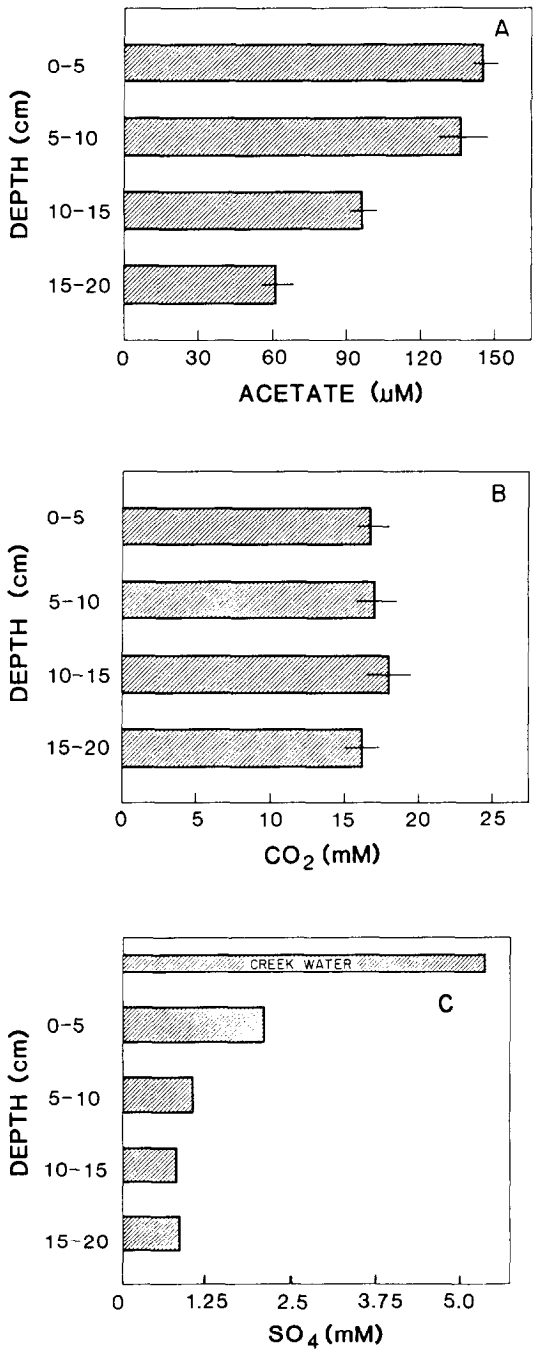

Fig. 4. Porewater concentrations of (*A*) acetate, (*B*) total dissolved CO_2 and (*C*) sulfate in 5 cm sections of site 2 (Shell Hammock) high marsh sediments. Sulfate concentrations are also shown for the adjacent creek. Error bars represent ± 2 SE of the mean (n = 3).

poration at each time point was as calculated using the equation:

$$\frac{\text{dpm in lipid carbon}}{T_n(N)} = \text{nanomoles lipid carbon per hour}$$

where T = time period (in hours) with substrate of specific activity n (dpm nM^{-1}) and N = specific activity of substrate (dpm nM^{-1}). Lipid carbon refers to radioactivity present in hydrophobic carbon compounds, i.e. cellular membrane lipids. Total lipid radioactivity in control cores injected with formaldehyde were less than 5% of experimental samples for both substrates and were not used to correct observed rates of synthesis. Lipid incorporation for $^{14}CO_2$ was based on its specific activity in sediment porewaters (0.412 nCi nM^{-1}) at time 0 and corrected for slight changes in concentration over the experimental period. Acetate specific activity was estimated over time from its specific activity in porewaters at time 0 (21.4 nCi nM^{-1}) minus any label appearing as $^{14}CO_2$ over the course of the incubation. The pattern of lipid incorporation was remarkably similar for both acetate and CO_2 with an initial pulse of lipid synthesis from the labelled

Table 1. Lipid incorporation from acetate and CO_2 in high marsh sediments (site 2, 10–15 cm layer).

Time (h)	Nanomoles lipid carbon per $cm^3 hr^{-1}$ (× 100)	Label used[2] (%)	Percent of total lipid radioactivity [1]				
			PL	FFA	Ether	ST	Other
1-^{14}C Acetate							
Avg. (± 1 sd)							
0.3	35.7 (0.2)	0.3	72.5	21.9	0	0	5.6
6	3.3 (0.5)	8.5	80.2	15.8	0	0	4.0*
12	2.1 (0.4)	8.9	88.0	8.3	0	0.8	2.9*
24	1.8 (0.3)	11.6	77.8	10.8*	tr	3.7	7.7
168	0.7 (0.2)	48.5	64.4	8.9	2.1	4.5*	19.8
$^{14}CO_2$							
1	169.7 (9.6)	0.1	84.6	12.3	0	0	3.1
24	7.7 (1.5)	0.14	79.5*	16.4	0	1.3	2.8*
48	8.7 (2.3)	0.30	78.1*	15.2*	tr	1.1*	5.6
72	6.1 (0.9)	0.37	70.6	19.6*	0.8	1.8*	6.9*
168	3.7 (0.5)	0.44	69.1*	15.3*	1.4*	3.9	10.3

[1] Lipids are shown as average percent of lipid radioactivity in phospholipids (PL), free fatty acids (FFA) sterols (ST) and ether lipids. Any values not significantly different ($p = 0.05$, $n = 2$) from the previous time point are designated by (*). tr = trace (< 0.5%).

[2] Percent label used equals the amount of radioactivity appearing as lipid divided by total added radioactivity for each substrate. Acetate calculations are corrected for any label appearing as $^{14}CO_2$, but should be considered qualitative.

pool followed by a more constant rate of accumulation which declined slightly over the experimental period. In incubations with both substrates, phospholipids accounted for the majority of the radioactivity incorporated into lipids, averaging 76.6% and 76.4% of the total lipid radioactivity from acetate and CO_2, respectively. The remaining lipid radioactivity consisted primarily of free fatty acids with small amounts of sterols and other neutral lipids. Of the total extracted lipid radioactivity present in 7-day acetate incubations, 2.1% contained the ether linkage as measured by differential hydrolysis whereas in CO_2 incubations 1.4% of the total lipid radioactivity was present as ether lipids. Sterols also appeared during the course of the incubation and increased to 4.5% of total lipid radioactivity by seven days. Since most bacteria do not contain sterols in significant amounts (Lechevaliar 1977) the late appearance of radiolabelled sterols are probably via routes such as grazing of labelled bacteria by microeukaryotic organisms.

Acetate oxidation & binding

The removal of ^{14}C acetate from porewater and its oxidation to $^{14}CO_2$ within intact sediment subcores is shown in Table 2. Calculation of acetate specific activity was estimated using the amount of label recovered over time assuming that the acetate porewater concentration at time 0 (96.2 μM) did not change. Correction was made for the appearence of $^{14}CO_2$ during the experimental period. Such measurements will therefore reflect only the specific activity of acetate free in sediment porewater and will exclude any fraction bound to sediment particles and not released by acidification. As shown in Table 2, acetate was rapidly removed from porewaters with less than one third of the injected label detectable twenty minutes after injection. The oxidation of acetate was also a rapid process with traces of $^{14}CO_2$ appearing

Table 2. Binding and oxidation of ^{14}C acetate in anoxic high marsh sediments.

Time (h)	Free acetate[1] (%)	Specific[2] Activity (nCi nM^{-1})	CO_2 as % of total acetate	CO_2 as % of free acetate
0	100	21.4	0	0
0.3	31.4	6.7	0.2	0.6
6	33.9	7.3	8.4	23.8
12	27.4	5.9	8.7	30.1
24	7.3	1.6	11.3	122.4

[1]Free acetate in porewater was calculated using the total radioactivity in aqueous samples after acidification and sparging.

[2]Acetate specific activity was based on label recovered in porewater minus $^{14}CO_2$ produced.

within 20 min of label injection and 11.3% of the total ^{14}C acetate injected present as $^{14}CO_2$ after 24 h.

Discussion

The range of methane production rates observed among the ten sites and four depths examined demonstrate the heterogeneity of methanogenesis in salt marsh sediments. Methane production rates spanned several orders of magnitude with the highest rates occurring in the high marsh sediments of Airport Marsh (site 1), and Shell Hammock (site 2), the areas most removed from tidal inundation. Although the rates of methane release from site 1 sediments in April were similar to those measured in August and September by King & Wiebe (1978), we did observe a pronounced increase in CH_4 production below the 0–5 cm horizon while they found no apparent peak in methane release within the top 11 cm. Of the 10 locations examined, it is interesting to note that while aerial (aboveground) production of *S. alterniflora* generally declines from creekbank to high marsh areas (Chalmers 1982), the opposite is observed for methane release, with high marsh sediments always showing increased rates compared to intermediate or creekbank sediments (Figs. 2, 3). This relationship is most apparent in transects A and B, where lower rates of methane release were present in the creekbanks and higher values in high marsh sediments. If carbon, energy and electron inputs to sediments follow an analogous pattern as input from *S. alterniflora* production, then factors other than substrate and energy availability may be more important determinants for methanogenesis in these sediments.

In most marine sediments the primary factor controlling methanogenesis is thought to be SO_4 availability and thus competition with sulfate reducing bacteria for both substrates and reductants such as H_2 (Mountford et al. 1980; Sorenson et al. 1981). In Shell Hammock (site 2) sediments where methanogenesis rates are comparable to published values in other sediments of high CH_4 production (Mountford et al. 1980; Crill & Martens 1983), porewater concentrations of acetate were always greater than 60 μM (Fig. 4a), and porewater CO_2 concentrations were never less than 16 mM (Fig. 4b). Since acetate and CO_2 represent two important substrates for methanogenesis, such high levels suggest that substrate concentrations alone do not limit CH_4 production in these sediments. Oremland et al. (1982) have suggested that competition with sulfate reducing bacteria may not be important in salt marsh sediments due to the utilization of "noncompetitive" substrates such as methanol and methylated amines. In such sediments,

however, the input of these substrates would be expected to decline with the decrease in plant production as one moves higher in the marsh. This is not the trend one might expect if "noncompetitive" substrate inputs were an important control for methanogenesis in marsh sediments. Rather, because sulfate depletion is usually greater in the high marsh (Howarth & Giblin 1983) competition for reductant with sulfate reducers is probably lessened, allowing the development of greater methanogenic activity. Among all sites and depths examined, CH_4 production was greater in 34 of 40 sediments with H_2 as compared with N_2 incubations and increased over ninefold in the 10–15 cm layer of site 1 sediments from 82 nanomoles CH_4 gram dry sediment^{-1} day^{-1} from cores incubated in a nitrogen atmosphere to 772 nanomoles of CH_4 when H_2 was substituted (Fig. 2). Such a major shift in methane production with the addition of H_2 suggests that an active population of methanogens was present whose growth was at least partially limited by the avilability of H_2. This does not exclude the utilization of "noncompetitive" substrates, which appear to be important in several marine sediments (King 1984), and may be responsible for the variability of H_2 in stimulating methane release and the low incorporation of acetate and CO_2 into ether lipids. In these salt marsh sediments, however, H_2 and SO_4 appear the primary factors controlling methanogenesis.

Although site 2 sediments showed substantial amounts of methane production, lipid synthesis measurements indicate that only small amounts of either substrate were incorporated into ether lipids typical of the methanogenic bacteria. The phospholipids, in contrast, where the dominant class of lipid synthesized throughout the experimental period (Table 1). The rapid synthesis of phospholipids has also been observed on detrital particles incubated for short periods in the presence of 1-^{14}C acetate (King et al. 1977) and with anaerobic sediments which were mixed and aerated (Moriarty et al. 1985). Although demonstrating that microbial communities in sediments have the ability for rapid uptake and synthesis of membrane lipids, the initial pulse in lipid synthesis which was observed may also be partially due to the injection technique employed. While the direct injection technique has the advantage of maintaining the spatial relationship in sediments, it may also deliver micropools or high substrate concentration relative to the whole sediment (Pomeroy & Wiegert 1981). Additional limitations have been discussed by Jones & Simon (1984) and as they note, incorporation rates of labelled compounds in sediments must consider the adsorption and diffusion of a particular compound. In the present work, the high rate of lipid synthesis seen over the first incubation period (0.3 and 1 h for acetate, and CO_2, respectively) could be due to incomplete substrate diffusion in sediments.

Similarly, the interpretation of lipid synthesis rates must also consider the oxidation of acetate and its binding to sediments. Christensen & Blackburn (1982) observed a rapid binding of injected acetate to sediment particles in Danish coastal sediments; in high marsh sediments we also observed the rapid disappearance of free ^{14}C acetate from sediment porewaters (Table 2). Microbial oxidation of both free and bound acetate must also occur since the amount of $^{14}CO_2$ in sediment porewaters after 24 h was significantly higher than the total recoverable acetate present in porewaters (Table 2). This process is particularly important in calculating rates of ether lipid synthesis which occur in the later stages of the experimental period. Because a large amount of acetate is rapidly bound, the synthesis of ether lipids between 24 and 168 h could arise from the slow release and exchange of bound acetate with the free pool, synthesis from an unknown metabolite, or CO_2 produced as a final oxidation product. While corrections can be made for the change in acetate specific activity as the experimental period proceeds, the rapid binding and oxidation of acetate from free, and bound pools, and the likely appearence of other metabolites does not allow us to determine the substrate for ether lipids as the incubation progresses. Until additional data is available, the synthesis of ether-linked lipids from acetate should be considered preliminary and thus viewed solely in a qualitative way.

An unexpected result was the ability of CO_2 to function as a significant precursor for cellular lipid synthesis in these high organic sediments (Table 1). The incorporation of CO_2 into lipids of the sediment community demonstrates that the flow of carbon is not to a single end product (CO_2 or CH_4) but that CO_2 can be reutilized as a source of cellular carbon. Several bacterial groups may be responsible for the autotrophic fixation of CO_2 we observed, including methanogenic and acetogenic bacteria. The utilization of CO_2 by acetogenic bacteria would also allow the secondary incorporation of CO_2 from acetate produced as an end product of acetogenic metabolism (Zeikus et al. 1985). Although the importance of acetogens in marine sediments is not clear, recent work by Jones & Simon (1985) indicates that acetogenic metabolism of CO_2 is an important source of acetate to lake sediments. In addition, heterotrophic bacteria could also contribute to the incorporation of CO_2 through anaplerotic reactions. In salt marsh sediments of Sapelo Island, a low proportion of organic nitrogen is present as a result of the input by *Spartina* detritus as the primary substrate (Pomeroy & Wiegert 1981). Under conditions of carbon availability but limited organic nitrogen, tricarboxylic acid cycle intermediates such as α-ketoglutarate and oxaloacetate will be diverted in heterotrophs to amino acid synthesis, thus promoting (anaplerotic) reactions such as the carboxylation of pyruvate

and phosphoenolpyruvate (PEP). This would result in the further incorporation of $^{14}CO_2$ into cellular lipids of the microbial community in addition to acetogenic and methanogenic activity. Our observations of CO_2 incorporation into lipids under conditions where oxygen dependent chemoautotrophic activity was absent (because of the long anaerobic incubations and the strongly reducing conditions) suggests that metabolic activity responsible for CO_2 incorporation in sediments cannot be easily attributed to a single microbial group. Observations of CO_2 incorporation in sediments must thus be carefully interpreted since the fixation of CO_2 in sediments does not appear to *a priori* imply chemoautotrophy (e.g. Kepkay & Novitsky 1980).

The predominance of phospholipids over the ether lipids throughout the experimental period in sediments of active methane production suggests that methanogenic bacteria play a small role in the flow of carbon as either acetate or CO_2 through microbial lipids (Table 1). Since methanogens can metabolize only a limited suite of substrates, acetate and CO_2 fixed into ether lipids should represent a substantial percentage of the carbon flow into lipids of the methanogenic bacteria (or other *Archaebacteria*) present. If one assumes a constant rate of synthesis, equilibration of all internal and external pools and that all carbon in ether linked lipids arise from the measured substrate pools, then ether lipid synthesis from acetate would equal 1.03×10^{-3} nanomoles lipid carbon per $cm^3 h^{-1}$ and synthesis from CO_2 0.9×10^{-3} nM lipid carbon per $cm^3 h^{-1}$. Although methanogenic bacteria vary in the molecular weight of the lipids they contain, (Kushwaha et al. 1981), they generally contain between 55 and 100 carbons per mole lipid. Using an average lipid content of 4% per cell (Tornabene & Langworthy 1978) and average weight of 1×10^{-13} grams $cell^{-1}$ (Bratbak 1985) a preliminary estimate of methanogen cell production based on lipid synthesis in salt marsh sediments from CO_2 and acetate can be calculated (Table 3).

These estimated production rates for methanogens suggest a relatively slow turnover of the methanogenic population in marsh sediments. From colony counts, Jones & Paynter (1980) estimated population densities of 10^3 to 10^6 cells gram dry $sediment^{-1}$, suggesting direct count values for methanogens of 10^6 to 10^9 cells gram dry $sediment^{-1}$ (Daley 1979). Our calculated

Table 3. Estimated methanogen production rates in anoxic salt marsh sediments[1].

	Nanomoles ether lipid carbon per $cm^3 day^{-1}$	Cells synthesized per cm^3 day^{-1}
1-^{14}C Acetate	11.9×10^{-2}	2.7×10^5
$^{14}CO_2$	6.5×10^{-2}	0.5×10^5

[1]See text for descrption of values used in the calculation. Production rates for acetate are shown for qualitative comparison only.

production of approximately 10^5 cells day^{-1} is equivalent to a doubling time of 7 (@ 10^6) to 7000 (@ 10^9) days. Other estimates of bacterial population turnover in these sediments (Weigert 1979; Fallon et al. 1983) also report relatively slow rates. Using a combination of techniques, Moriarty et al. (1985) concluded that methanogens are minor consumers of carbon and energy in seagrass beds, with sulfate reduction accounting for a high proportion of organic matter decomposition. In these anoxic salt marsh sediments, rates of methane release demonstrate that methanogen are an active component of the sediment microflora. The results of lipid synthesis measurements (the predominance of phospholipid synthesis and late appearence of ether lipids), however, suggest that while catabolic processes may be active, lipid biosynthesis and thus cell turnover by the methanogenic population is a minor process in comparison with other members of the microbial community.

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