Nitrogen Fixation in Arctic Marine Sediments

R. KNOWLES

Technical Report No. 9

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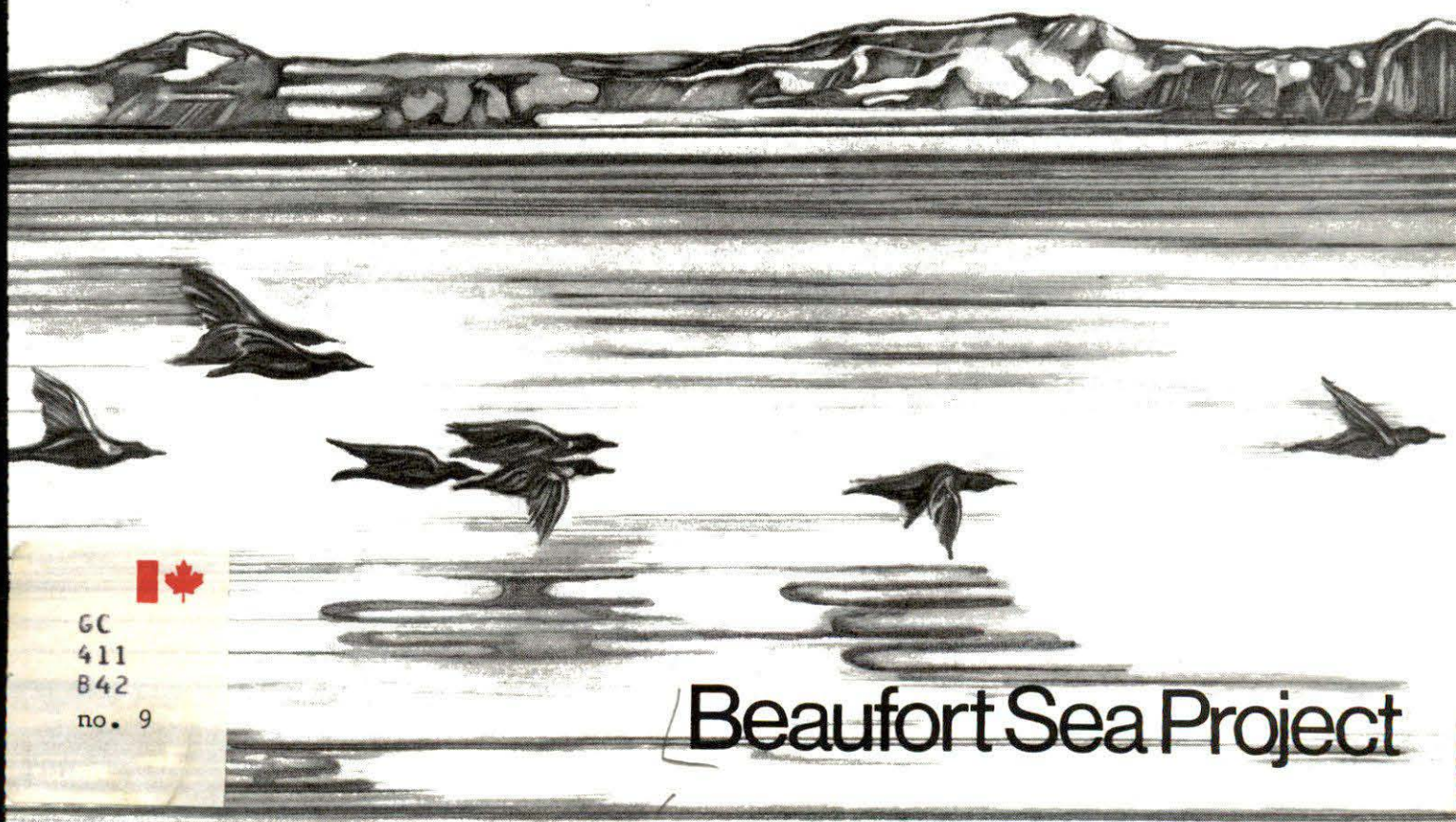

Beaufort Sea Project

NITROGEN FIXATION IN ARCTIC MARINE SEDIMENTS

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1. SUMMARY

Nitrogen fixation was measured in grab and core samples of sediments from the Beaufort Sea and Eskimo Lakes. The indirect assay involving the reduction of acetylene to ethylene was used. Very low rates, of the order of 25 mg N/m^2. year, were detected in untreated sediments. Activity was markedly stimulated by addition of glucose, sucrose, lactose, mannitol and malate, much less by acetate, and negligible activity was supported by N-acetylglucosamine, the chitin monomer. There was no consistent effect of the presence or absence of oxygen and evidence suggested that anaerobic and facultatively anaerobic bacteria were responsible for the observed activities. Nitrogen fixation potentials in glucose-supplemented sediment samples showed large variation between stations, between samples from the same station, and between depths within single cores down to 18 cm. Consequently experimental reproducibility and replicability was poor.

Denitrification of nitrate to the gaseous products N_2O and N_2 was not detected in untreated sediments but was potentially very active when sediments were supplemented with organic carbon and nitrate. Reduction of the nitrate followed the sequence $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$.

Weathered Norman Wells crude oil, decane, dodecane and hexadecane had no effect, stimulatory or inhibitory, on nitrogen fixation, denitrification or carbon dioxide evolution. Hexane caused partial inhibition of denitrification but not of nitrogen fixation, and 1,2,4-trimethylbenzene caused complete inhibition of both nitrogen fixation and denitrification but only partial inhibition of CO_2 evolution. There was no evidence of utilization of any of the hydrocarbons tested during periods of over 30 days under the experimental conditions employed.

2. INTRODUCTION

2.1. Nature and Scope of Study

The importance of biological nitrogen fixation in the biogeochemical cycle has long been recognized. Although great progress has been made in recent years in the study of the biochemistry, physiology, and certain aspects of the ecology of the process, few studies have been made of nitrogen fixation in sediments. The potential contribution to the nitrogen economy of marine systems, particularly those of the arctic, is unknown.

With increasing development of petroleum exploration in the arctic marine environment it has become imperative that baseline studies begin. The present work was conducted using sediment grab and core samples from the Beaufort Sea and Eskimo Lakes (N.W.T.). It was essentially a laboratory study of a sediment model system. Effects of oil and of certain petroleum fractions on nitrogen fixation, and, to a small extent denitrification were investigated.

2.2. Objectives

The specific objectives are listed as follows:

a. Obtain grab and core samples of selected regions of the inshore areas of the Beaufort Sea and of estuarine areas of the Eskimo Lakes, through the cooperation of staff of the Arctic Biological Station.

b. Perform indirect assays of N_2 fixation using the acetylene-ethylene technique and study the microbial populations responsible for the observed activity.

c. Study the variations of N_2-fixing activity or of potential activity with season, area and depth in the sediment.

d. Study the effect of such factors as availability of different energy and carbon substrates, and presence of oil or petroleum fractions on sediment N_2 fixation.

e. Study, if time and facilities permit, the occurrence of denitrification (reduction of nitrate via nitrous oxide to N_2) in relation to the above factors.

2.3. Relation to Offshore Drilling

Data provide preliminary baseline information from which it is possible to speculate on the potential impact of spills from drilling operations on some of the sediment nitrogen transformations which are themselves important for the maintenance of marine biological productivity in the arctic.

3. CURRENT STATE OF KNOWLEDGE

3.1. Nitrogen-fixing Bacteria in Marine Sediments

Marine sediments have been shown to contain N_2-fixing bacteria of the following genera:
Azotobacter (Bavendamm, 1932; Waksman, Hotchkiss and Carey, 1933; Pshenin, 1963).
Enterobacter (Werner, Evans and Seidler, 1974).
Clostridium (Waksman, Hotchiss and Carey, 1933; Pshenin, 1963; Patriquin and Knowles, 1972).
Methanobacterium (Barker, 1940).
Desulfovibrio (Sisler and Zobell, 1951; Trüper, Kelleher and Jannasch, 1969; Patriquin and Knowles, 1972).
Chlorobium (Buchanan and Gibbons, 1974).
Most of the above organisms are obligately or facultatively anaerobic and thus, in view of the anaerobic nature of many sediments and the provision of energy sources in the plankton rain, it would appear that a potential for N_2 fixation exists. Such a process would contribute, through sediment-water exchange, to the nutrient levels of the marine water column. However, it must be remembered that close to the aerobic-anaerobic sediment interface denitrification may also occur, resulting in a loss of nitrogen in gaseous form (N_2O and/or N_2).

3.2. Nitrogen Fixation in Marine Sediments

Actual estimates of N_2 fixation in marine sediments, however, are very few. Brooks *et al.* (1971) demonstrated nitrogenase activity in the 2 to 5 cm depth in estuarine mud in Florida, and Bunt *et al.* (1971) measured low activities also in sediments of Card Sound, Florida. Patriquin and Knowles (1972) showed the existence of nitrogenase activity in a wave-disturbed skeletal carbonate sand from Barbados. The latter activity was greatly stimulated by substances released by roots of marine angiosperms or by the addition of glucose or mannitol (Patriquin and Knowles, 1975). Maruyama, Suzuki and Otobe (1974) also showed low rates of N_2 fixation in Pacific near shore and offshore sediments and these activities were stimulated by the addition of glucose, mannitol, sucrose and pyruvate.

3.3. Nitrogen Fixation in Fresh-water Sediments

In fresh-water lake sediments N_2 fixation was shown to make sometimes significant contributions (Howard *et al.* 1971; Toetz, 1973) and in Lake Mendota, Wisconsin, to account for 5 to 8 per cent of the total annual combined nitrogen input (Macgregor *et al.* 1973).

3.4. Nitrogen Fixation in Arctic Systems

A review of the IBP Tundra Biome Circumpolar Study of Nitrogen Fixation (Alexander, 1974) attributed most activity in terrestrial

or semiterrestrial systems to blue-green algae either free-living or as a component of lichens. Heterotrophic bacteria were implicated by several workers. However, there are no reports on arctic sediments.

3.5. Hydrocarbons and Nitrogen-fixing Bacteria

Methane-utilizing nitrogen-fixing bacteria have been isolated and have received considerable attention (Pine and Barker, 1954; Davies *et al.*, 1964; Coty, 1967; Whittenbury *et al.*, 1970; DeBont and Mulder, 1974).

The report by Coty (1967) of the isolation of nitrogen fixers able to utilize butane, tetradecane, toluene, and cyclohexanecarboxylic acid was not subsequently followed up or confirmed. However, Rivière *et al.* (1974) reported nitrogen fixation by bacteria which utilized hexadecane but unfortunately they did not present any data from acetylene or ^{15}N assays.

The possible effect of petroleum on nitrogen fixation does not appear to have been studied in any system.

3.6. Denitrification in Sediments

Physiological and some ecological aspects of denitrification of nitrate to gaseous products by soils and sediments were reviewed by Pichinoty (1973), Payne (1973) and Garcia (1975). Denitrification was active in fresh water sediments (Chen *et al.*, 1972) and in the water column of a subarctic lake during the anoxic winter period (Goering and Dugdale, 1966), and was also detected in anaerobic marine sediments off Peru (Goering and Pamatmat, 1971). There appear to be no studies of this process in arctic marine sediments.

4. STUDY AREAS

Samples of sediment were taken by Arctic Biological Station personnel from stations (Fig. 1) in the Beaufort Sea and the Eskimo Lakes. Details are given in Appendix Tables 1 and 2.

Stations were Beaufort Sea 7, 9, 12, 14, 22, 23, 29, 30, 544-559, 555, 556 and 558; Eskimo Lakes 508, 510, 515, 520, 523 and Clupea Harbour (550). Some samples were obtained for preliminary study in Feb. 27, 1974 and the remaining samples were obtained between May 24, 1974 and August, 1975 (Appendix, Tables 1 and 2).

5. METHODS

5.1. Types of Sediment Samples

Samples were of two types:
Core samples were taken using a polycarbonate (cellulose acetate butyrate) plastic liner 38 mm outside diameter, 1.5 mm wall thickness and 270 mm long used inside a Phleger corer. The actual sedi-

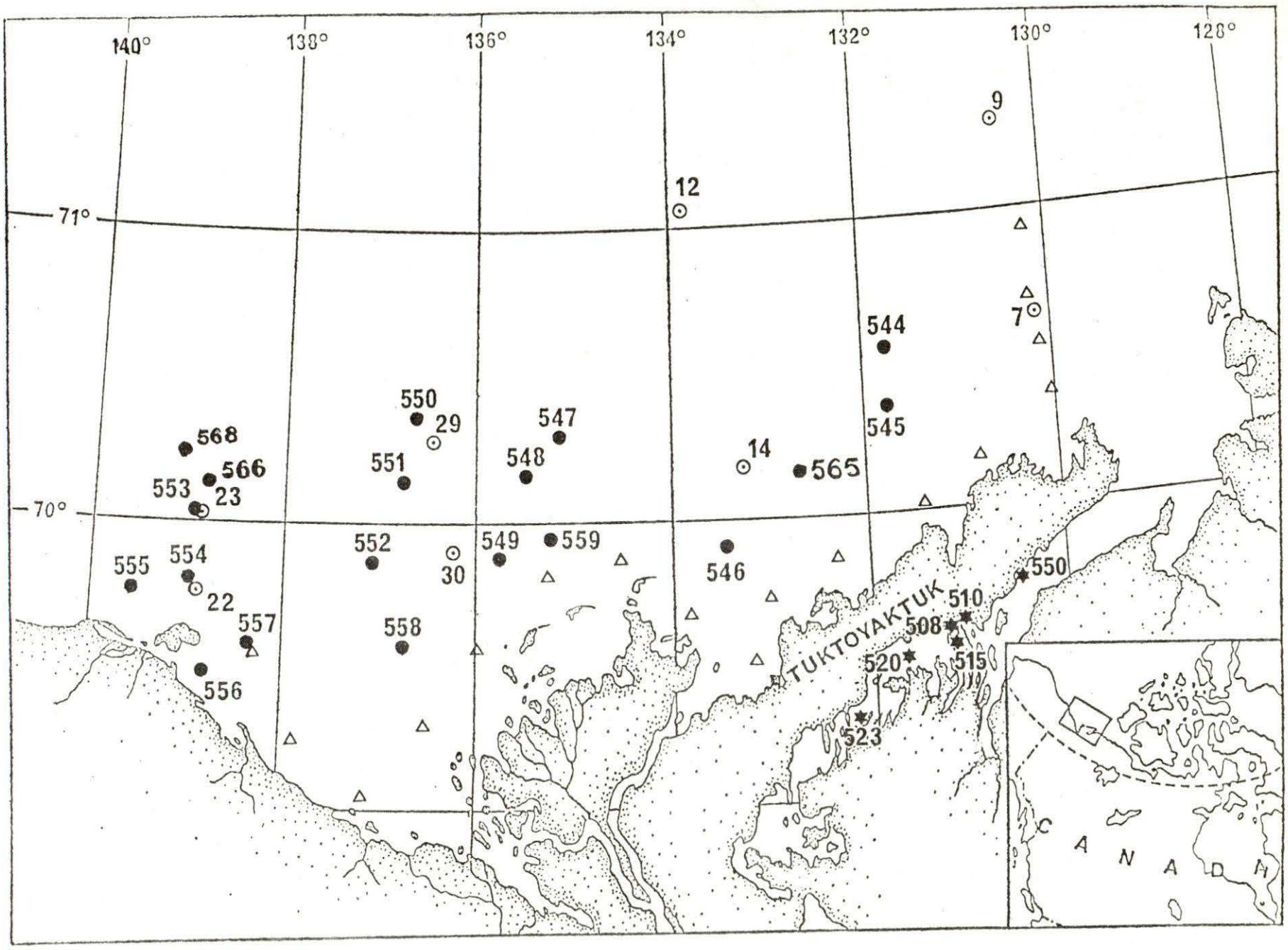

Fig. 1. Locations of sampling stations in the Beaufort Sea and Eskimo Lakes.

ment cores were usually 11 to 20 cm in length. Rubber stoppers (size 8) were inserted and taped in place.
Grab samples of approximately 1 to 3 kg wet weight were taken using a Petersen Grab and then transferred to gas-impermeable plastic bags.

5.2. Handling and Storage of Samples

Sediment samples were handled in three different ways: (a) Worked on almost immediately in the laboratory at Inuvik, N.W.T., by Mr. Ross Harland (Arctic Biological Station), (b) Packed in low-temperature equilibrated polystyrene Trans-Temp containers and shipped to the Montreal laboratory (temperature control by this method was about 4 to 12°C). (c) Packed with dry ice in polystyrene containers and shipped in the frozen state to the Montreal Laboratory. On receipt, the grab samples were transferred to plastic jars and, together with the cores, stored at 4° C.

5.3. Materials

Samples of unweathered and weathered Norman Wells crude oil and of several petroleum fractions (hexane, decane, dodecane, hexadecane and 1,2,4-trimethylbenzene) were supplied by Dr. J.N Bunch, Arctic Biological Station, Ste. Anne de Bellevue.

Supplies of helium, nitrogen, hydrogen and air were from Liquid Carbonic Canadian Corporation, and of acetylene, ethylene and nitrous oxide were from Matheson Company Ltd.

Chemicals were obtained from Fisher Scientific Company and Canadian Laboratory Supplies Ltd.

5.4. Sediment Model System

A ten-gram sample of wet sediment (approximately 4 to 6 g dry weight) was introduced, by means of a cut-off 5-ml disposable syringe, into the bottom of each of a series of 50-ml Erlenmeyer flasks. Nutrients were added, when necessary, in 0.5 ml of artificial seawater (Patriquin and Knowles, 1972) of the following composition: NaCl 0.3 M; $MgSO_4$ 0.05 M; $CaCl_2$ 0.01 M; KCl 0.01 M; Tris 0.05 M; adjusted to pH 7.5. For Eskimo Lakes sediments half the above concentrations were used. Glucose, mannitol, lactose and N-acetylglucosamine were added to give 0.5% w/w final concentration. Sodium acetate was added at an equivalent concentration on a carbon basis.

In the study of cores a plunger was used to push the sediment from the tube. The sediment was then cut into one-centimeter sections and processed as above.

Each flask was closed with a Suba Seal rubber serum stopper. The atmosphere in the flask was left aerobic, or, if anaerobic conditions were required, the flask was evacuated and back-filled with

N_2 (or with He in denitrification experiments) three times to one atmosphere. When necessary, 10% of the gas phase volume was withdrawn by syringe and replaced with an equal volume of acetylene (C_2H_2) to give 0.1 atm pC_2H_2.

Throughout the above procedures every attempt was made to keep the sediment cold using crushed ice and refrigerator and cold room facilities. Experimental flasks were usually set up in triplicate and, unless otherwise indicated, were incubated at 15° C in the dark.

5.5. Analytical

Samples (usually 0.5 ml) of the gas phase were taken, at different times after injection of C_2H_2, and were analyzed by gas chromatography for C_2H_2 and for the ethylene (C_2H_4) produced by reduction of the C_2H_2 by N_2-fixing microorganisms. The gas chromatography system consisted of a column (200 cm x 3 mm) of 80- to 100-mesh Poracil C/methyl isocyanate at 45° C with N_2 carrier at 14 ml/min and an H_2 flame ionization detector.

Gas phase samples (0.5 ml) were also analyzed for O_2, N_2 and CO_2 using a Fisher-Hamilton Gas Partitioner Model 29, having a column (183 cm x 6 mm) of 30% DEHS on 60- to 80-mesh chromosorb P followed by a column (198 cm x 5 mm) of 40- to 60-mesh molecular sieve 13x at ambient temperature. Helium carrier flow was 40ml/min with a thermal conductivity detector (Brouzes, Mayfield and Knowles, 1971).

Anaerobic atmospheres were analyzed for N_2, N_2O, CO_2 and CH_4 by gas chromatography using a column (274 cm x 4.8 mm) of 80- to 100-mesh Porapak Q at 35° C with He carrier at 36 ml/min flow rate and a thermal conductivity detector (Knowles and Denike, 1974).

Data are the means of triplicates or duplicates (except in core studies), and, unless otherwise stated, are on a sediment dry weight basis.

5.6. Inuvik Experiment

One experiment was carried out at the Inuvik laboratory. Acetylene was generated from calcium carbide (CaC_2). Gas samples (0.5 ml) were injected into 5-ml Vacutainers and shipped to the Macdonald Campus for analysis. Data were corrected for the small amount of leakage occurring during storage of Vacutainers.

5.7. Populations of Nitrogen-fixing Bacteria

Certain plate count and Most Probable Number (MPN) estimates were made of microbial populations. The details of the media and

methods used were as described by Patriquin and Knowles (1972). The plate count of total aerobic heterotrophic bacteria used Difco Tryptic Soy Broth solidified with Oxoid Ionagar. The MPN estimates of facultatively anaerobic N_2 fixers utilized a sucrose + mineral salts + growth factor medium, and sets of tubes were incubated aerobically and anaerobically. The MPN estimates of N_2-fixing clostridia utilized a glucose + acetate + mineral salts + phenosafranin + soil extract medium incubated anaerobically, and the MPN estimates of sulfate-reducing bacteria (many of which fix N_2) used a lactate + yeast extract + mineral salts + steel wool medium incubated anaerobically.

5.8. Phasing of the Work

Sampling procedures in the arctic were such that frequently many samples were received at the laboratory in one shipment. Since the experimental procedures involved were very time-consuming it was not possible to study many samples at the same time. This necessitated refrigeration storage of samples until such time as they could be worked on.

6. RESULTS

It was anticipated that there would be problems because of the inherent heterogeneity of the sediment samples and the difficulty or impossibility of homogenizing the material without disturbing conditions within the sediment. Reproducibility did in fact turn out to be poor as is illustrated later, particularly in the studies of core samples.

6.1. Effect of Shipping Conditions

Different portions of a mixed grab sample from Eskimo Lakes station 508 were used for routine C_2H_2 reduction assays (a) on freshly collected sediment (experiment performed at Inuvik) and on sediment after shipping to Montreal (b) chilled in Trans-temp containers or (c) frozen with dry ice. The data in Table 1 show a very small C_2H_4 production by samples incubated in the absence of C_2H_2 (whether supplemented with glucose or not), and somewhat greater C_2H_4 production when C_2H_2 was present but without a glucose supplement. The addition of glucose markedly stimulated reduction of C_2H_2 (nitrogenase activity) after a lag period of 48 to 96 hours. In the aerobic series there was no great difference in activity between fresh, chilled and frozen samples. In this and in other experiments there was no consistent difference in activity between samples shipped chilled and those shipped in the frozen condition (see also Fig. 2 later).

TABLE 1. Maximum rates of production of C_2H_4 (nmoles C_2H_4/g·day) by sediment from Eskimo Lakes station 508 (grab sample).

Shipment	Presence of				Incubation conditions	
	0.1 atm	pC_2H_2	Glucose		Aerobic	Anaerobic
None, fresh*		-	-		0.3	-
		-	+		0.5	-
		+	-		0.9	-
		+	+		182	-
Chilled		+	+		120	250
Frozen		+	+		129	173

* These sediment samples were assayed at the Inuvik laboratory.

These data suggested that it was not necessary to conduct all experiments on fresh samples at Inuvik and that shipment in the chilled or frozen state was a valid procedure to permit studies in the Montreal laboratory.

6.2. Utilization of Organic Carbon and Energy Sources

Table 2 shows the results of experiments to test the ability of several carbon compounds to support C_2H_2 reduction activity in the sediment. Glucose, sucrose, lactose, mannitol, and sodium malate all supported appreciable activity. Sodium acetate, however, did so only after a considerable time lag and the chitin monomer N-acetylglucosamine supported negligible activity. In subsequent experiments, therefore, glucose was used routinely as a supplement.

TABLE 2. Maximum rates of production of C_2H_4 (n moles C_2H_4/g·day) from C_2H_2 by sediment from Eskimo Lakes station 508 (grab sample) supplemented with various organic compounds (C content equivalent to that of 0.5% w/w glucose).

Organic C supplement	Aerobic	Anaerobic	
	Exp. 1	Exp. 1	Exp. 2
None	-	-	0.2
Glucose	962	517	197
Sucrose	-	-	146
Lactose	-	-	150
Mannitol	757	-	73
Na acetate	8	-	78*
Na malate	-	-	133
Nacetylglucosamine	6	9	1.0

* A lag period of 7 days was observed. In all other cases lags of 1 to 2 days occurred.

6.3. Effect of Incubation Temperature and Presence or Absence of Oxygen

Table 3 shows the effects of temperature and of glucose supplement on activity. Both in the presence and in the absence of oxygen extremely low and roughly similar activities were observed in the absence of glucose and at 5° C. Addition of glucose at 15° C permitted significant activity, illustrating the existence of a nitrogen fixation potential. In Tables 1, 2 and 3 there are no consistent differences between the activities observed under initially aerobic conditions and those observed under anaerobic conditions. This may not be unexpected in view of the probable establishment of a significant anaerobic zone in sediment below a thin aerobic interface.

The pattern of consumption of O_2 and production of CO_2 by glucose-amended sediment is illustrated in Fig. 2. Thus "aerobic" samples contained as much as 40 per cent of the O_2 initially present until about 10 days of incubation. Thus an aerobic surface zone could be expected to persist for at least this period of time. Production of CO_2 was much greater under aerobic than under anaerobic conditions. However, in view of the pH and carbonate equilibria in the sediments the CO_2 production data in

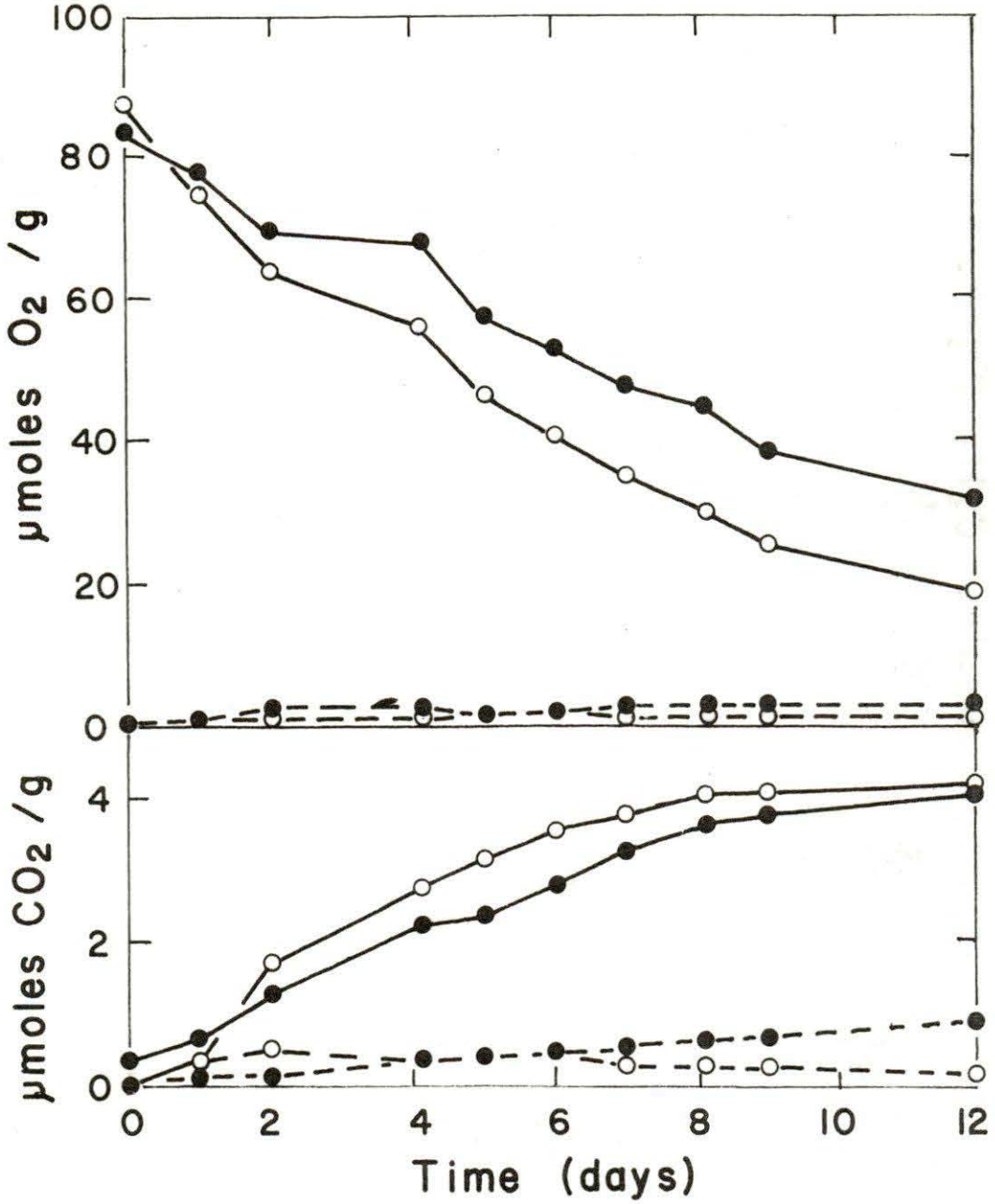

Fig. 2. Concentrations of O_2 and CO_2 in flasks containing sediment from a grab sample taken at Eskimo Lakes station 508. Samples were shipped chilled (closed circles) or frozen (open circles), then supplemented with 0.5% glucose and incubated at 15°C in closed flasks aerobically (solid lines) or anaerobically (broken lines).

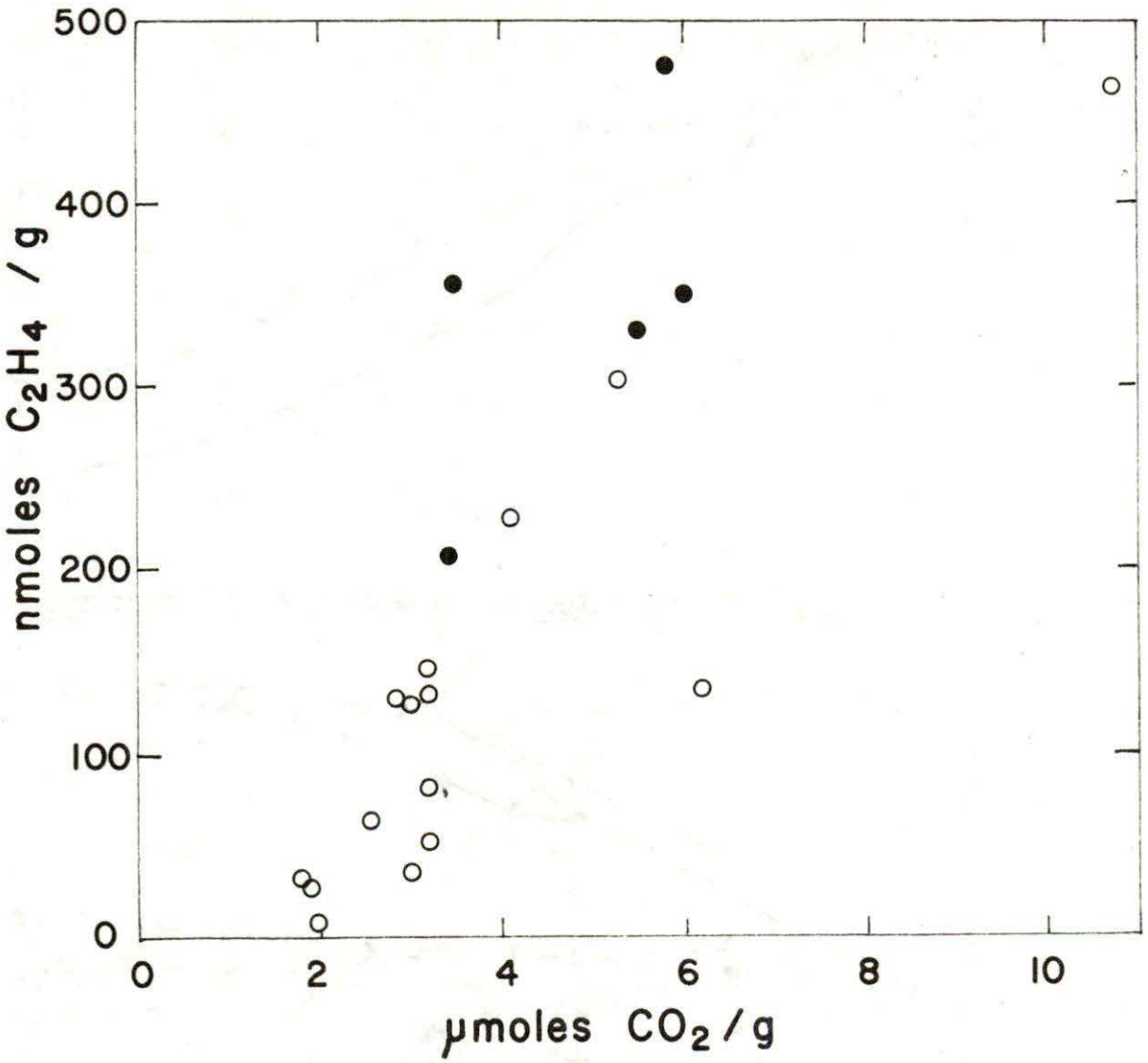

Fig. 3. Production of C_2H_4 from C_2H_2 and evolution of CO_2 during 132 hours of anaerobic incubation of glucose-supplemented sediment samples from different stations in the Beaufort Sea (open circles) and Eskimo Lakes (closed circles).

in this report are meaningful only in relative terms and do not indicate absolute rates.

TABLE 3. Rate of production of C_2H_4 (nmoles C_2H_4/g·day) from C_2H_2 by sediment from Eskimo Lakes station 508 (grab sample).

Supplement	Incubation conditions			
	Aerobic		Anaerobic	
	5°C	15°C	5°C	15°C
None	0.1 - 0.3	0.1 - 0.7	0.1 - 0.3	0.1 - 1.0
Glucose	0.1 - 0.5	10 - 700	0.1 - 0.6	140 - 500

Values shown are ranges of activities observed with a series of chilled and of frozen samples.

6.4. Potential Activity at Different Stations

Sediment grab samples from 20 different stations in the Beaufort Sea and Eskimo Lakes were supplemented with glucose and incubated anaerobically with C_2H_2 for 132 hours, at which time the quantities of C_2H_4 and CO_2 were determined. The amounts of C_2H_4 and CO_2 produced thus give rough comparative indications of potential N_2 fixation and carbon metabolism in the sediments. These data, along with the pH values of the sediment are given in Appendix Table 3. Figure 3 shows the rough correlation observed between C_2H_4 production and CO_2 production in the samples. The Eskimo Lakes sediments (closed circles) showed generally greater activities, and the isolated highly active Beaufort Sea sediment sample was from station 545 in the region of the MacKenzie River plume.

6.5. Potential Activities of Core Segments

In these experiments cores of sediment from Beaufort Sea station 14 and Eskimo Lakes station 508 were cut into 1-cm deep sections. During extrusion of the cores there was some compression of the sediment and therefore the core depths recorded are nominal rather than accurate. Each section was placed in a separate flask and the production of C_2H_4 from C_2H_2 as well as the production of CO_2 was followed. The rates shown in Figures 4 to 12 are those observed during the phase of active C_2H_2 reduction after the usual lag period of approximately 24 to 72 hours. They thus might be termed potential or maximum activities.

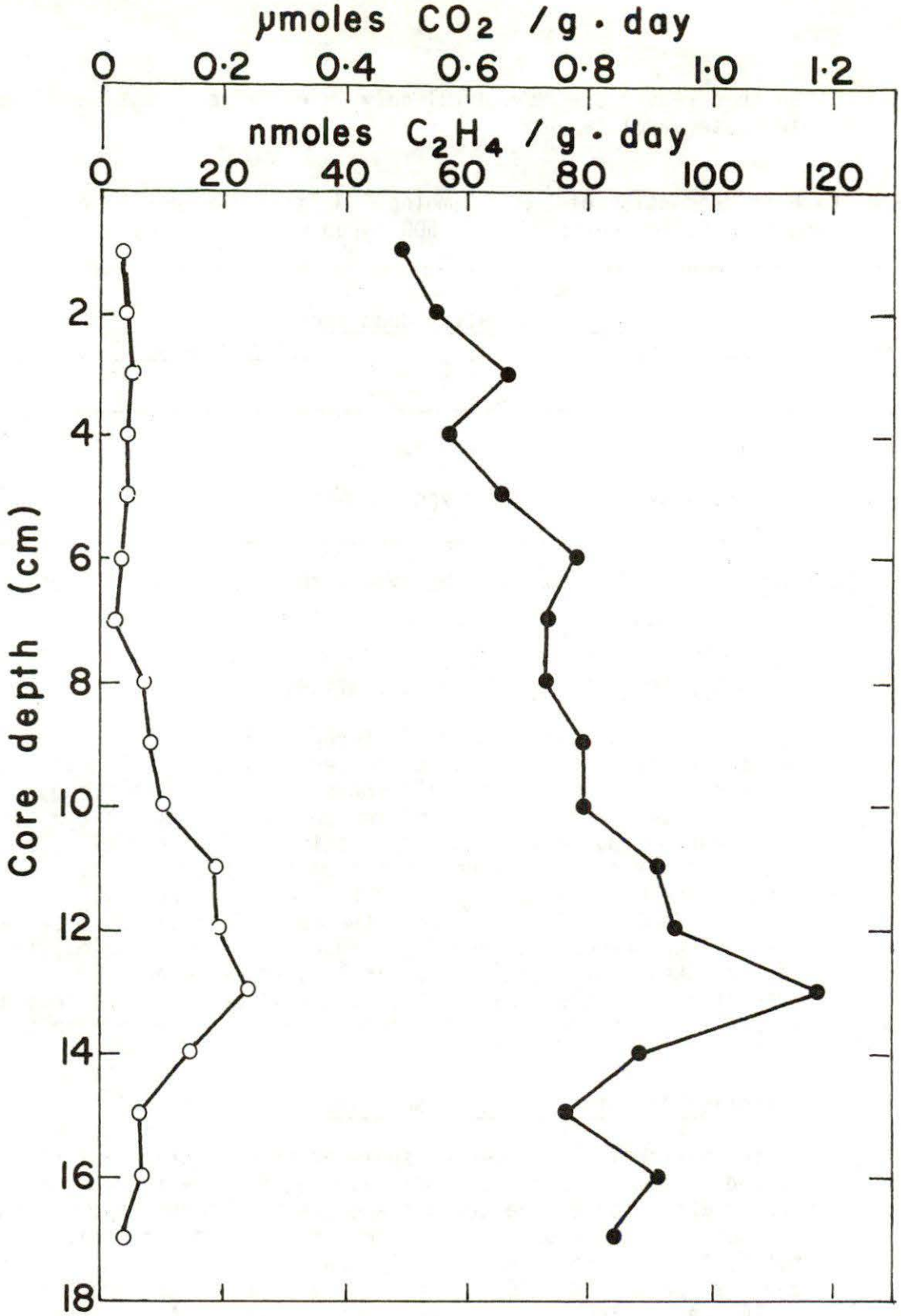
μmoles CO2 / g · day
0
0·2
0·4
0·6
0·8
1·0
1·2
nmoles C2H4 / g · day
0
20
40
60
80
100
120
Core depth (cm)
2
4
6
8
10
12
14
16
18

Figure 4

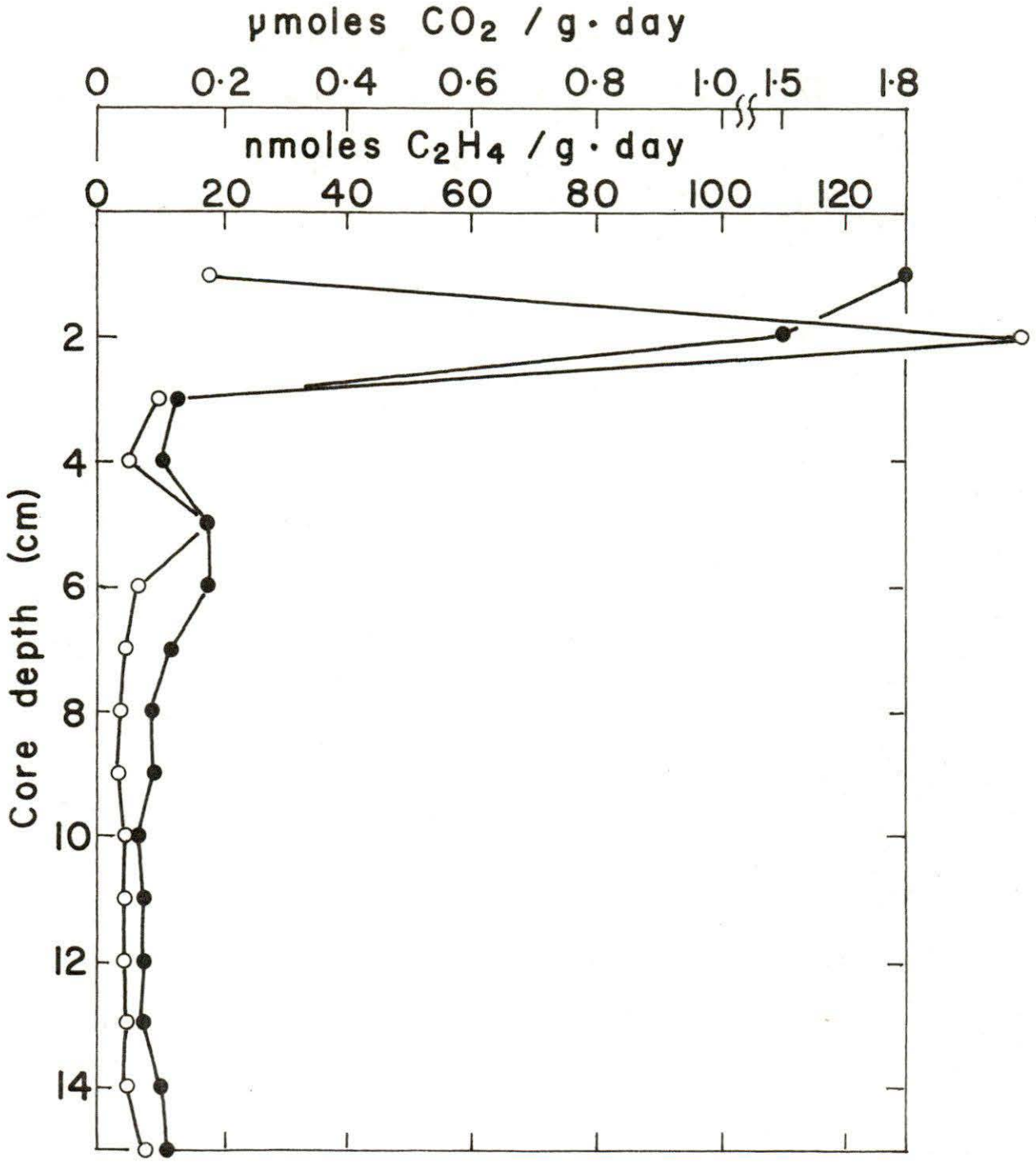

Fig. 5. Same as Fig. 4. Data for a second core, also aerobic incubation.

Fig. 4. (Opposite page) Maximum rates of production of C_2H_4 from C_2H_2 (open circles) and evolution of CO_2 (closed circles) by sediment sections from a core taken at Beaufort Sea station 14. Sediment was supplemented with glucose and incubated at 15°C under an initially aerobic atmosphere.

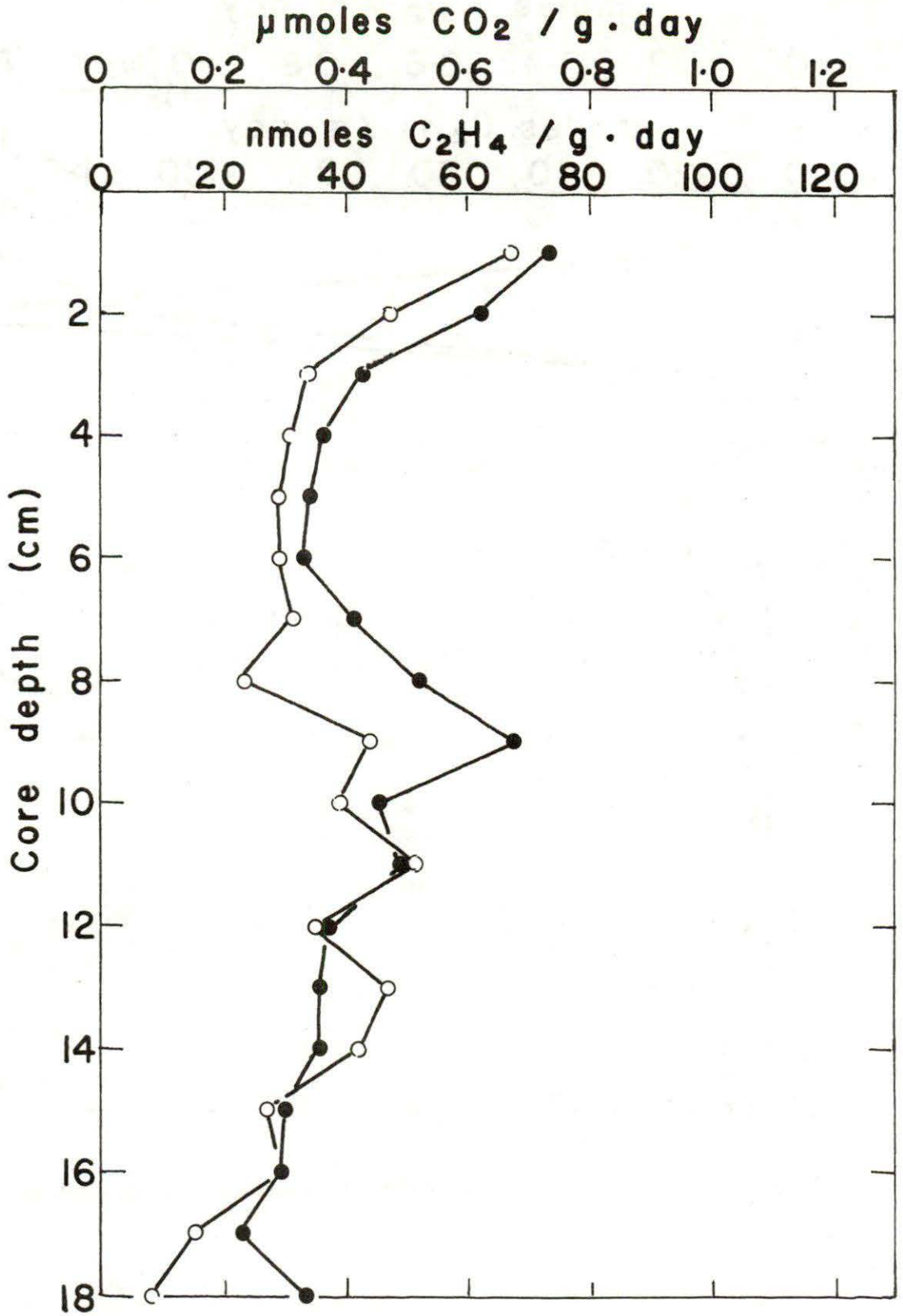
μmoles CO2 / g · day
0
0·2
0·4
0·6
0·8
1·0
1·2
nmoles C2H4 / g · day
0
20
40
60
80
100
120
Core depth (cm)
2
4
6
8
10
12
14
16
18

Figure 6

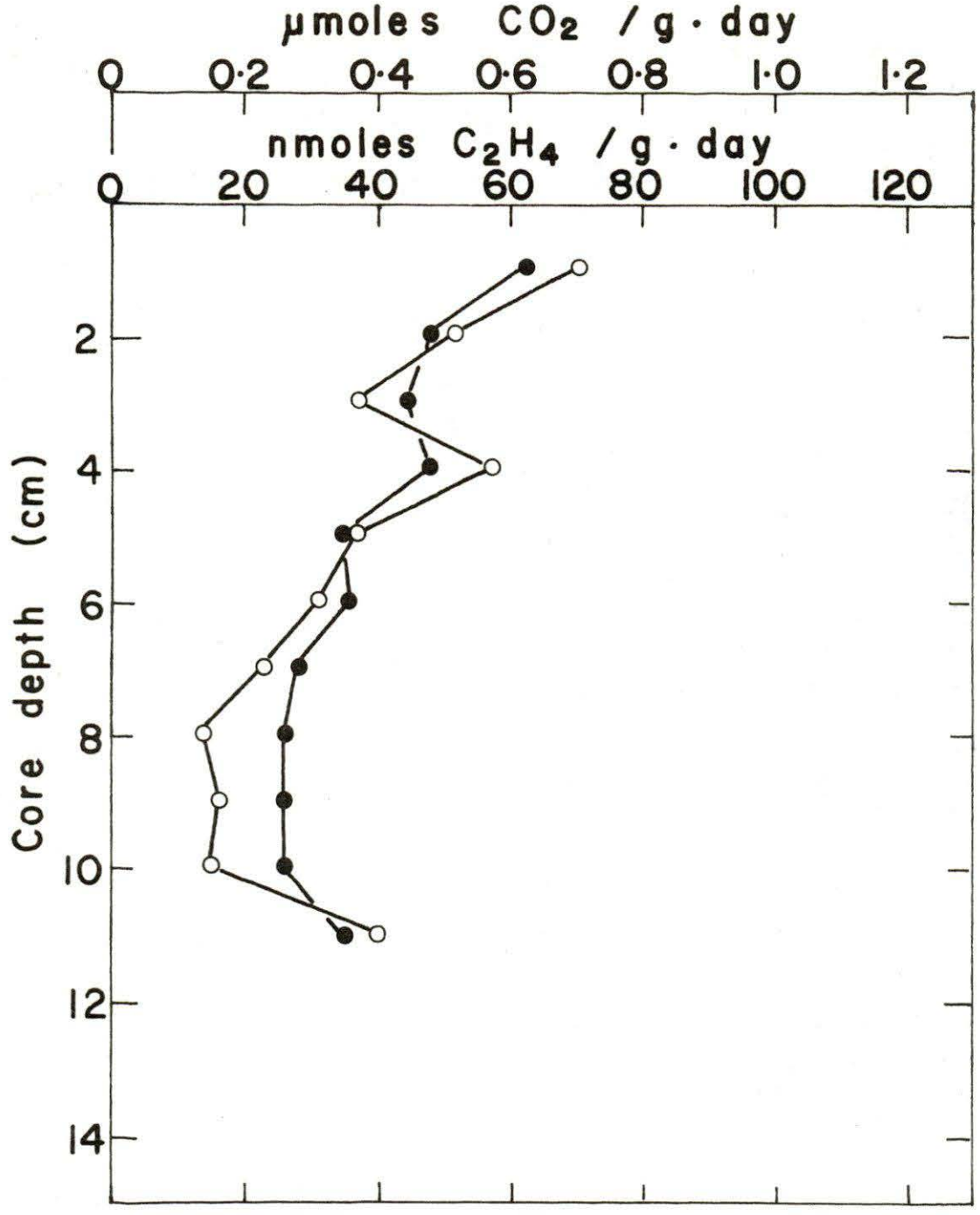

Fig. 7. Same as Fig. 6. Data for a second core, also anaerobic incubation.

Fig. 6. (Opposite page) Maximum rates of production of C_2H_4 from C_2H_2 (open circles) and evolution of CO_2 (closed circles) by sediment sections from a core taken at Beaufort Sea station 14. Sediment was supplemented with glucose and incubated at 15°C under an anaerobic atmosphere.

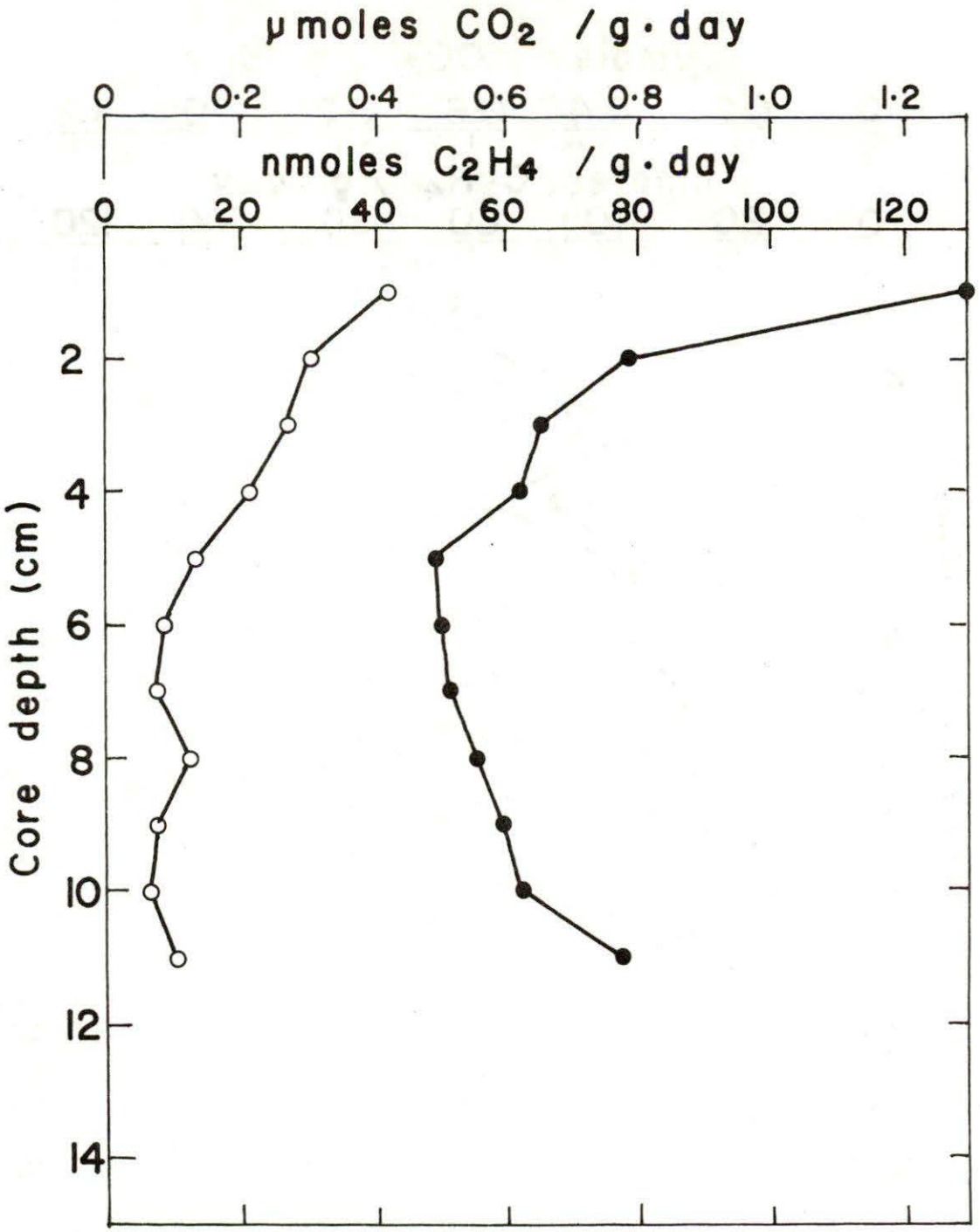

Fig. 8. Maximum rates of production of C_2H_4 from C_2H_2 (open circles) and evolution of CO_2 (closed circles) by sediment sections from a core taken at Eskimo Lakes station 508. Sediment was supplemented with glucose and incubated at 15°C under an initially aerobic atmosphere.

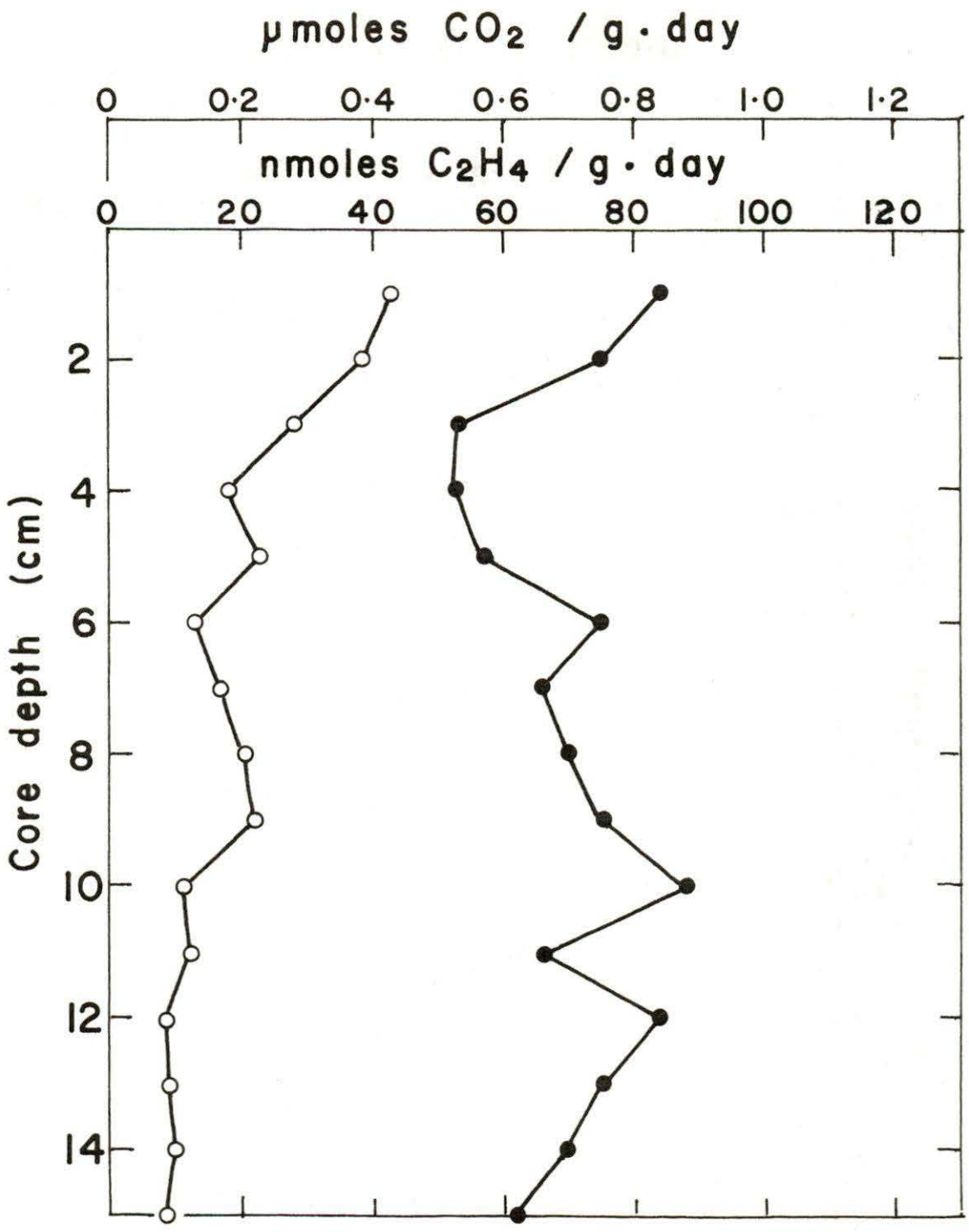

Fig. 9. Same as Fig. 8. Data for a second core, also aerobic incubation.

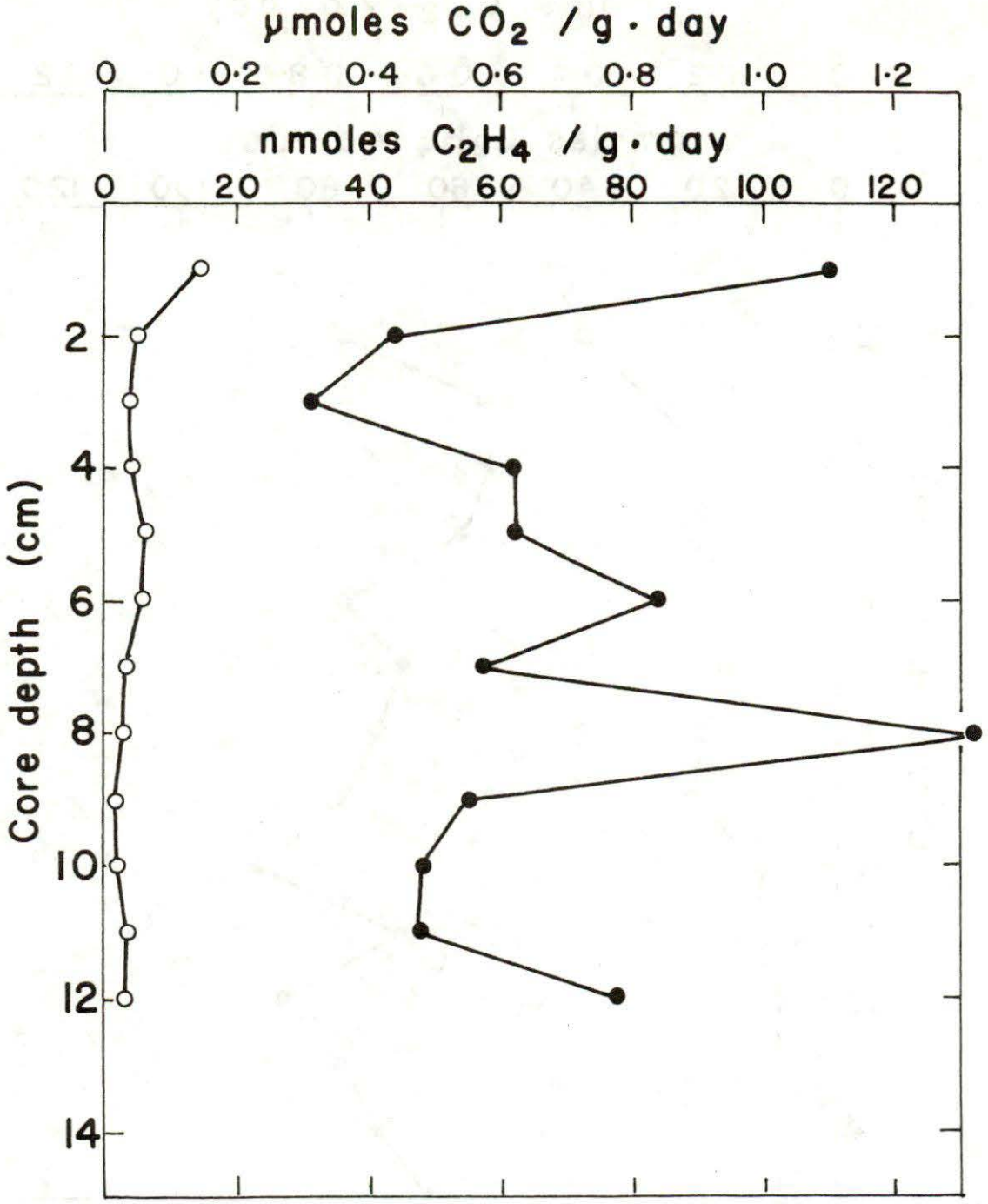

Fig. 10. Same as Fig. 8. Data for a third core, also aerobic incubation.

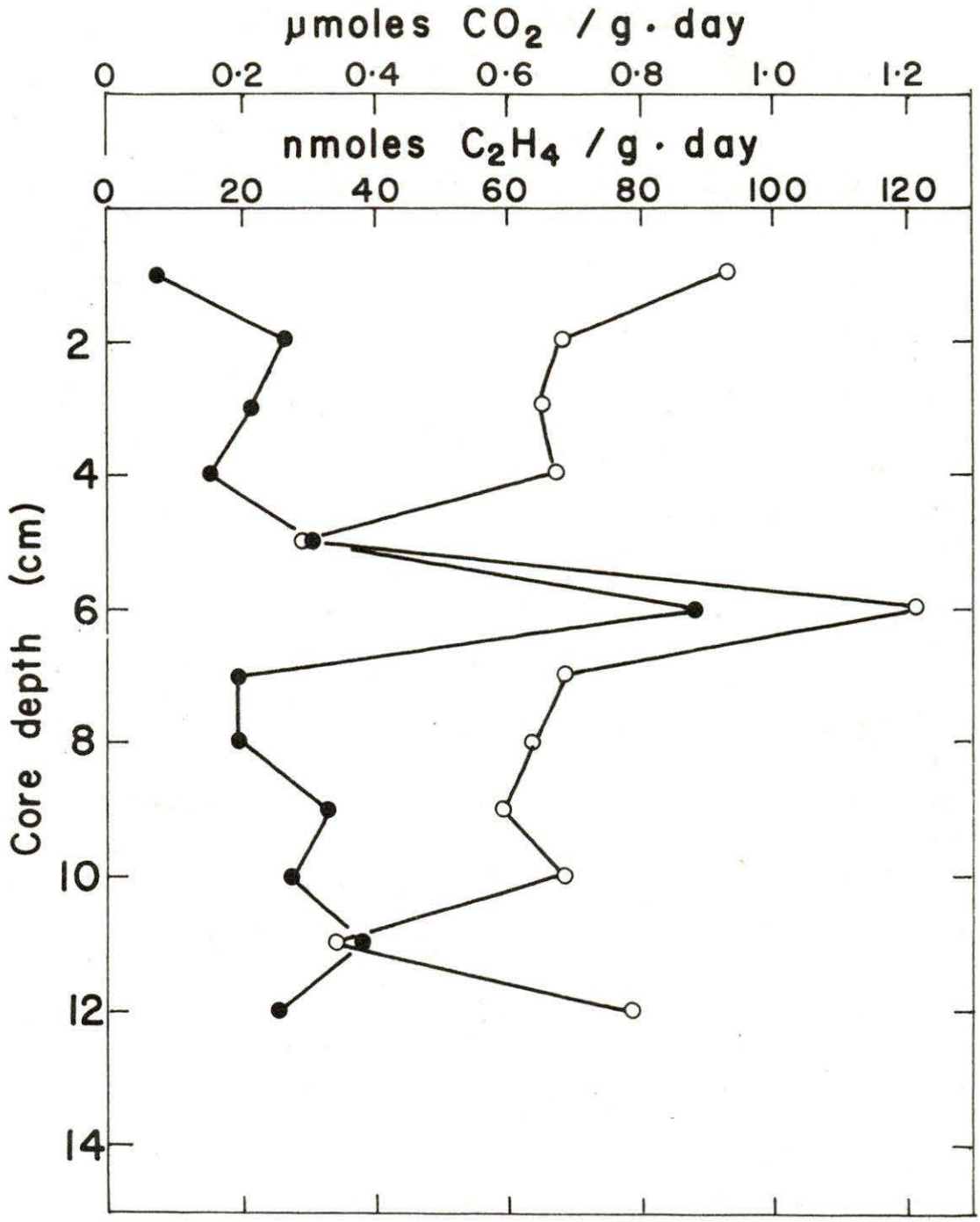

Fig. 11. Maximum rates of production of C_2H_4 from C_2H_2 (open circles) and evolution of CO_2 (closed circles) by sediment sections from a core taken at Eskimo Lakes station 508. Sediment was supplemented with glucose and incubated at 15°C under an anaerobic atmosphere.

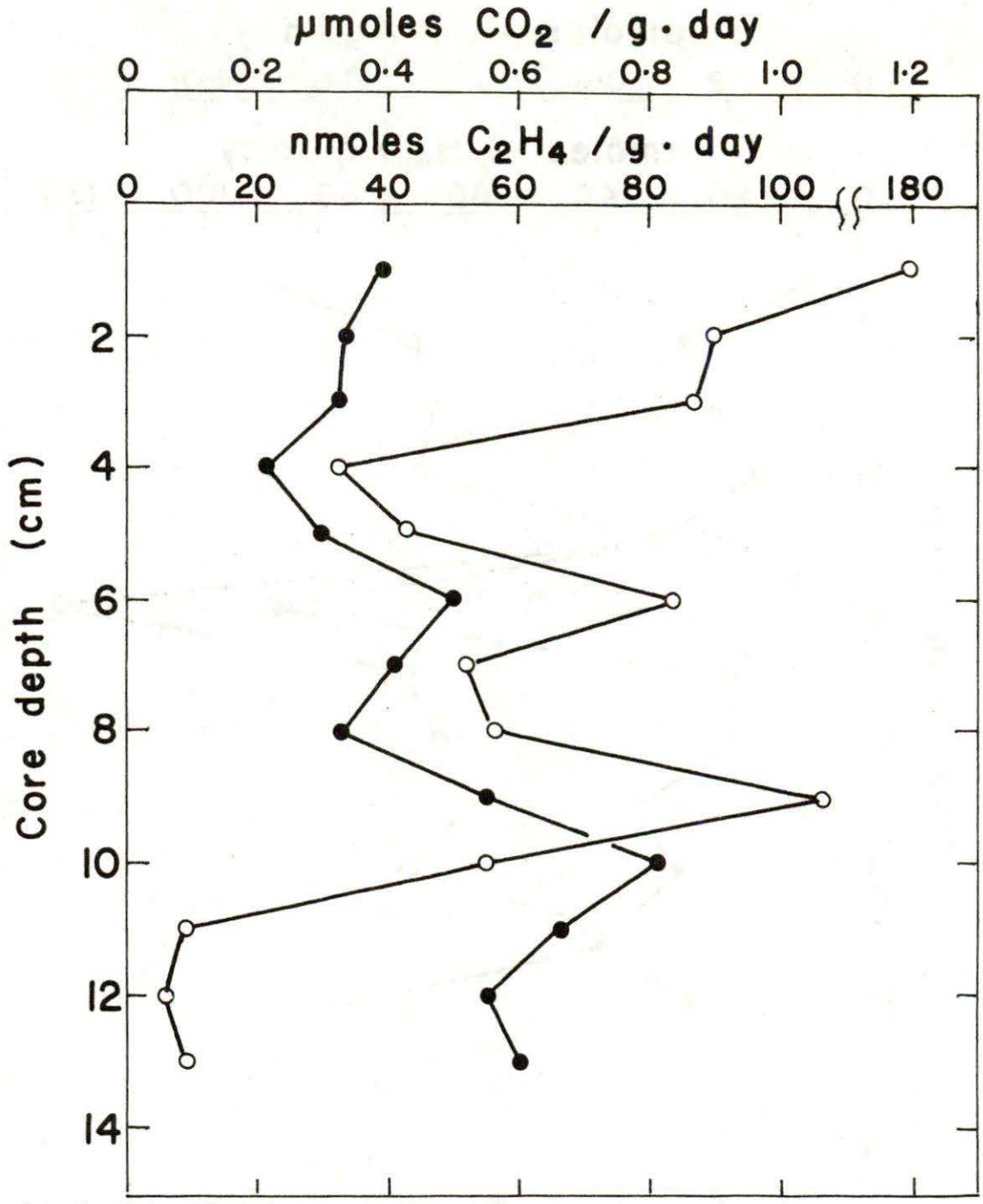

Fig. 12. Same as Fig. 11. Data for a second core, also anaerobic incubation.

Figures 4 to 7 represent Beaufort Sea sediment (two aerobic and two anaerobic) and Figures 8 to 12 represent Eskimo Lakes sediment (three aerobic and two anaerobic).

It is clear that there were large variations in activity not only between cores but also with depth in each core. The profile of activity with core depth was frequently very pronounced and often differed greatly between two cores taken from the same station (see for example Figs. 4 and 5). Depth profiles were more or less smooth except in the anaerobically incubated Eskimo Lakes sediment (Figs. 11 and 12).

An important feature of the data was the relatively close relationship which existed between each profile of C_2H_4 production and the corresponding profile of CO_2 production. Variations between cores and between depths were no doubt due to qualitative and quantitative differences in microbial populations as well as in availability of nutrients.

TABLE 4. Maximum rates of production of C_2H_4 (nmoles/g·day) and CO_2 (μmoles/g·day) by sediment from Eskimo Lakes in the presence of varying quantitities of weathered Norman Wells crude oil.

			Milliliters oil per 10 g sediment					
	Addition		0		0.1		0.3	
	Glucose	C_2H_2	C_2H_4	CO_2	C_2H_4	CO_2	C_2H_4	CO_2
Exp. 1*	-	-	0	0.29	0	0.43	0	0.49
	-	+	0.17	0.27	0.41	0.44	0.33	0.53
	+	-	0	1.68	0	1.61	0	1.63
	+	+	99	0.71	90	0.75	81	0.74
Exp. 2	+	+	163	4.4	100	2.8	86	3.3
Exp. 3	-	+	0.26	0.18	0.45	0.11	0.45	0.01
	+	+	173	0.32	198	0.23	228	0.80

* Exp. 1. Eskimo Lakes station 515 sediment, aerobic incubation, 16 days.
Exp. 2. Eskimo Lakes station 508 sediment, aerobic incubation, 30 days.
Exp. 3. Eskimo Lakes station 508 sediment, anaerobic incubation, 28 days.

6.6. Effect of Oil and Hydrocarbon Fractions

In preliminary experiments it was found that volatiles released from crude oil (Norman Wells) interfered with the gas chromatographic measurements involved in the C_2H_2 reduction assay. Subsequent experiments were therefore conducted using weathered Norman Wells crude oil.

Table 4 shows results of three experiments in which different amounts of crude oil were added to sediment in the presence and in the absence of various combinations of glucose and C_2H_2. In the absence of C_2H_2 there was negligible production of C_2H_4 regardless of oil supplement. In the absence of glucose low rates of reduction of C_2H_2 to C_2H_4 occurred and these were slightly stimulated by oil, although in Experiment 3 there was no evidence of any stimulation of CO_2 production - indeed a decrease was observed. In the presence of glucose rates of production of C_2H_4 and CO_2 were much greater but there was no evidence of a consistent effect, stimulatory or inhibitory.

TABLE 5. Maximum rates of production of C_2H_4 (nmoles/g·day) and CO_2 (μmoles/g·day) by sediment from Eskimo Lakes station 508 in the presence of varying quantities of weathered Norman Wells crude oil, of hexane and of decane under anaerobic incubation.

Addition		Milliliters oil or oil fraction per 10 g sediment					
		0		0.1		0.5	
Oil etc.	Glucose	C_2H_4	CO_2	C_2H_4	CO_2	C_2H_4	CO_2
Oil	-	0.52	0.04	0.85	0.01	0.45	0.02
	+	393	1.13	381	0.98	330	0.88
Hexane	-			0.39	0.03	0.92	0.03
	+			381	1.21	359	1.09
Decane	-			0.19	0.01	0.86	0.02
	+			476	0.90	379	1.03

Duration of experiment was 35 days.

Results from a similar experiment in which weathered crude oil, hexane and decane were compared are shown in Table 5. Again there were no consistent effects of any of the materials on sediment either alone or when supplemented with glucose. The data on CO_2 production suggested no utilization of the hydrocarbons by the sediment microflora during the 35 day duration of the experiment.

Table 6 shows data from a similar experiment in which the possible effects of hexane, dodecane, hexadecane and 1,2,4-trimethylbenzene were compared. The first three compounds showed no effect on C_2H_4 or CO_2 production. Trimethylbenzene, however, completely inhibited C_2H_4 production and partially inhibited production of CO_2 from glucose. It appeared slightly to stimulate production of CO_2 from sediment in the absence of glucose but the significance of the difference between this and the other treatments is rather dubious.

TABLE 6. Maximum rates of production of C_2H_4 (nmoles/g·day) and CO_2 (μmoles/g·day) by sediment from Eskimo Lakes station 508 in the presence of various petroleum fractions (0.3 ml/10 g sediment) under anaerobic conditions for 24 days.

Fraction added	No glucose		Plus glucose	
	C_2H_4	CO_2	C_2H_4	CO_2
None	0.6	0.08	203	0.7
Hexane	0.6	0.10	197	0.7
Dodecane	0.8	0.08	172	0.6
Hexadecane	0.6	0.07	167	0.5
1,2,4-trimethyl-benzene	0.1	0.21	0	0.18

In this experiment, after making each flask anaerobic the sediment was mixed by "Vortex" agitation.

6.7. Populations of Nitrogen-fixing Bacteria

Attempts to estimate the population sizes of nitrogen-fixing bacteria in the sediments were limited to two stations (Beaufort Sea 565 and Eskimo Lakes 515) and the results are shown in Table 7. In the three successive estimates on Eskimo Lakes sediment the variability was pronounced, as was also the variability in activity estimates reported earlier. Indeed it is difficult to conclude very much from the few data available except that the N_2-fixing bacteria present probably include at least three groups: facultatively anaerobic organisms such as *Bacillus*, *Enterobacter* and *Klebsiella*, obligately anaerobic bacteria such

as *Clostridium* and sulfate-reducing N_2 fixers of the *Desulfovibrio* group. The data also suggest that populations may be smaller in the Beaufort Sea sediments but further studies are necessary.

TABLE 7. Numbers of bacteria per gram of dry sediment.

Group	Eskimo Lakes 515 Exp. 1	Eskimo Lakes 515 Exp. 2	Eskimo Lakes 515 Exp. 3	Beaufort Sea 565
Plate count: total aerobic heterotrophs	40,000	15,200	-	-
MPN counts:				
Facultative N_2 fixers				
anaerobic tubes	650	2,800	28,000	2,600
aerobic tubes	2,800	12,800	260	900
N_2-fixing clostridia	-	≥6,600	28,000	2,000
SO_4-reducers	1,400	≥6,600	1,250	86

6.8. Significance of Nitrogen Fixation in Arctic Marine Sediments

Based on the limited number of measurements of nitrogenase activity in sediments not supplemented with any source of carbon and energy (for example, see Table 3) it is possible to calculate an extrapolation to nitrogen fixation in these sediments on an area basis. Table 8 lists the data and assumptions used in this extrapolation and shows that N_2 fixation can amount to 25 mg $N/m^2 \cdot$year or 255 g N/ha·year. In the Discussion section this value is compared to other reports in the literature.

TABLE 8. Calculation of significance of nitrogen fixation in unamended arctic marine sediments. Parameters and assumptions used.

Activities (from Table 3)	5°C	0.1 - 0.3 nmoles C_2H_4 /g·day
	15°C	0.1 - 1.0 nmoles C_2H_4 /g·day
	Assume	0.1 nmoles C_2H_4 /g·day
Dry weight (ave. of 20 stations)		0.54 g dry wt/g fresh wt
		0.75 g dry wt/ml sediment
Theoretical C_2H_4/N_2 conversion factor		3
Assume 10 cm active sediment depth		
Assume temperature close to 5°C for 365 day/year		
Therefore N_2 fixation = 25 mg N/m^2·year		
= 255 g N/ha·year		

6.9. Denitrification in Arctic Marine Sediments

Certain bacteria, besides being able to use oxygen as a terminal electron acceptor, can also use oxidized nitrogen compounds for this purpose. When this process involves the reduction of nitrate (NO_3^-) with the eventual release of gaseous products, nitrous oxide (N_2O) and nitrogen (N_2), it is termed denitrification and is in fact the most important mechanism by which combined nitrogen is returned to the atmospheric N_2 reservoir. The sequence of reductions is as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

It was not possible to detect denitrification in sediments which were not supplemented with glucose or nitrate. However, the addition of these materials permitted denitrification to occur as can be seen in Table 9. In this experiment the relatively high concentration of NO_3^- added caused accumulation of NO_2^- which evidently in turn inhibited further metabolism. The products N_2O and N_2 accumulated in the ratio 1:2.

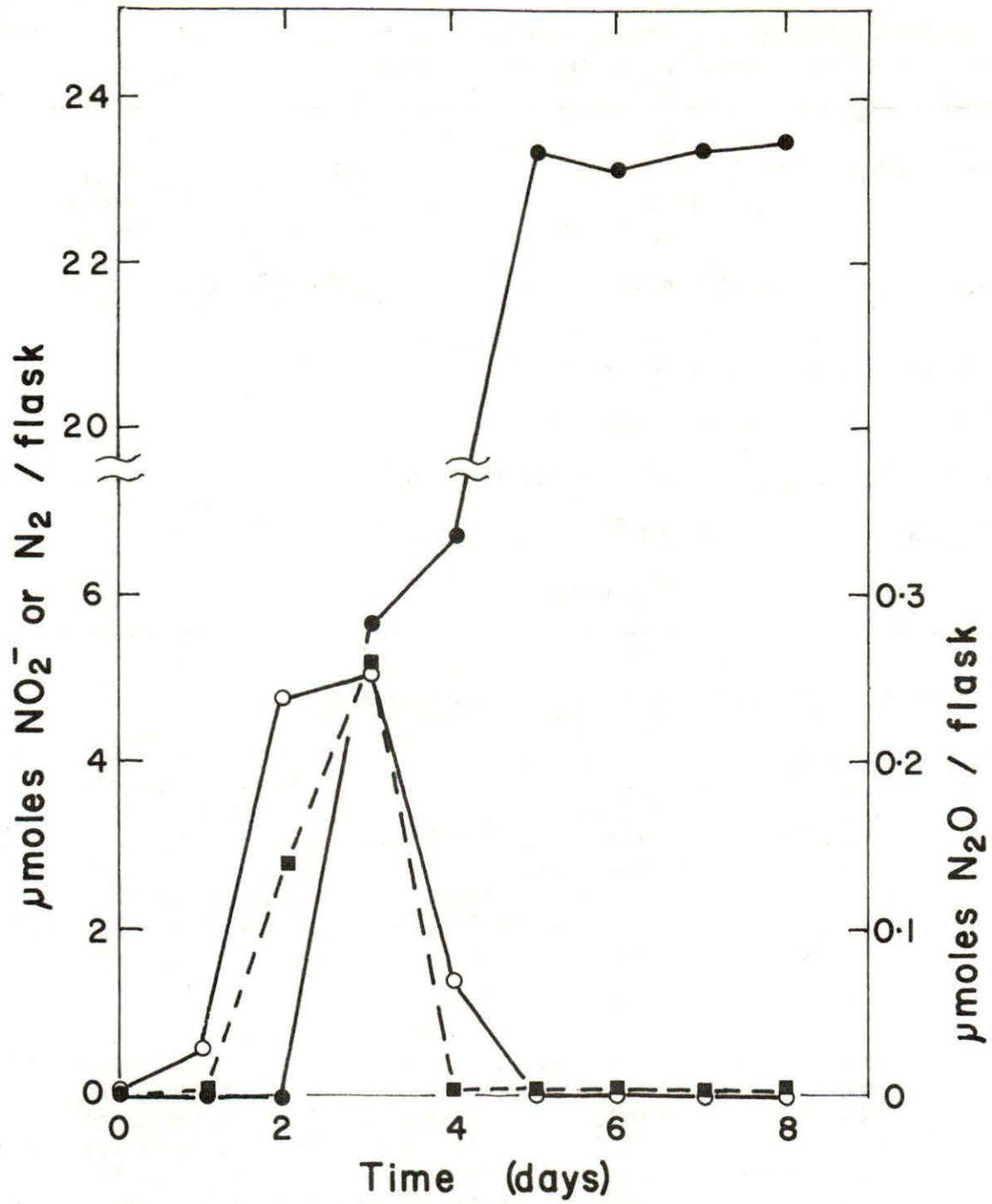

Fig. 13. Production of NO_2^- (open circles), N_2O (closed squares) and N_2 (closed circles) from added NO_3^- by sediment from Eskimo Lakes station 508. Ten grams wet weight of sediment received 50 μmoles $NaNO_3$ and 750 μmoles glucose and was incubated anaerobically.

TABLE 9. Transformation of added nitrate in sediment from Eskimo Lakes station 508 supplemented with glucose and incubated anaerobically. Data are μmoles per flask.

Days	NO_3^-	NO_2^-	N_2O	N_2	Glucose	CO_2
0	348	0	0	9	722	1
5	150	187	3.3	41	365	113
10	100	155	24.7	48	310	105

Ten grams wet weight (approx. 5 g dry wt) of sediment received 500 μmoles $NaNO_3$ and 750 μmoles glucose (about 2.7% w/w).

Figure 13 shows the results of a similar experiment in which the sediment was supplemented with glucose and only 50 μmoles of $NaNO_3$ per flask. In this system there was transient accumulation of NO_2^- and N_2O and the further reduction of these compounds was coincident with the appearance of N_2 to a final amount almost equal to that of the nitrogen added as NO_3^-.

In a further experiment (Table 10) in which 250 μmoles $NaNO_3$ were added per flask the presence of slowly solubilizable forms of copper and manganese appeared to promote somewhat the further reduction of NO_2^- and N_2O to N_2.

TABLE 10. Transformation of added nitrate in sediment from Eskimo Lakes station 515 supplemented with glucose in the presence and in the absence of copper and manganese and incubated anaerobically. Data are μmoles per flask.

Addition	Days	NO_3^-	NO_2^-	N_2O	N_2	Glucose	CO_2	Cu^{++}	Mn^{++}
None	0	330	0	0	4	1 000	2	0.08	46
	9	26	80	11	9	860	72	0.06	84
	14	24	20	11	8	850	86	0.11	102
CuO	0	330	0	0	4	1 000	2	0.15	43
	9	31	60	22	9	860	43	0.67	68
	14	28	30	9	17	860	49	1.10	69
MnO_2	0	330	0	0	3	1 000	2	0.07	45
	9	19	20	9	12	780	66	0.07	87
	14	20	12	0.5	27	780	158	0.10	116

Seven grams wet weight (approx. 3.5 g dry wt) of sediment received 250 μmoles $NaNO_3$, 1500 μmoles glucose and 10 mg CuO or 10 mg MnO_2 as indicated.

The effect of weathered Norman Wells crude oil and of several petroleum fractions on the production of N_2O from NO_3^- by sediment was studied in the experiment recorded in Table 11. Again about 20 to 30 μmoles N_2O was produced in about 10 days and this activity was not affected by crude oil, dodecane or hexadecane. However, N_2O production was markedly inhibited by hexane and was completely inhibited by trimethylbenzene, an effect apparently analogous to its effect on acetylene reduction reported in section 6.6. Under the conditions of the experiment described in Table 11 it was not possible to detect N_2O production by sediment in the absence of added nitrate and glucose. Further studies using more sensitive methods will be necessary in order to evaluate the possible occurrence of denitrification in unsupplemented sediments so as to permit extrapolation to *in situ* conditions.

TABLE 11. Production if nitrous oxide (N_2O) from nitrate by sediment from Eskimo Lakes station 515 supplemented with glucose under anaerobic incubation. Data are μmoles per flask.

Addition	Days of incubation				
	0	3	7	10	14
None	0	0.4	2.7	22	39
Oil 0.1 ml	0	0.6	3.8	37	40
0.5 ml	0	0.8	3.8	37	45
Hexane 0.1 ml	0	0.1	1.1	2	4
Dodecane 0.1 ml	0	0.4	2.4	24	38
Hexadecane 0.1 ml	0	0.1	3.7	44	54
Trimethylbenzene 0.1 ml	0	0	0	0	0
None, minus $NaNO_3$	0	0	0	0	0
None, minus $NaNO_3$ minus glucose	0	0	0	0	0

Seven grams wet weight (approx. 3.5 g dry wt) of sediment received 250 μmoles $NaNO_3$, 1500 μmoles glucose, 250 μmoles NH_4Cl, 100 μmoles sodium phosphate buffer (pH 7.4), and weathered Norman Wells crude oil or petroleum fractions as indicated.

7. DISCUSSION

7.1. Nitrogen Fixation

7.1.1. Variation

The variation of activity observed on an area basis (between cores or between grabs) and with depth in the sediment indicates the kind of problem which exists in the study of samples collected by grab. Here the sample is subjected to rather haphazard mixing and horizons of differing potential activity are redistributed in the partially mixed sample. It may be necessary to devise some method of homogenization without aeration, to permit the carrying out of adequately replicated experiments. The degree of spatial variation observed could prevent the detection of seasonal variation in actual or potential activity, even supposing a complete series of samples could be obtained.

7.1.2. In Situ Rates

The actual activities observed in sediments not supplemented with glucose were very low indeed and in Table 12 they are compared to results of similar measurements reported in the literature for other systems. Thus the rates reported here are of the same order as was observed in sediments from a Wisconsin lake (Macgregor *et al.*, 1973), Barbados (Patriquin and Knowles, 1972), and Tokyo Bay (Maruyama *et al.*, 1974), but, as might be expected, are lower than those reported from the other studies mentioned in the Table. A contribution of 25 mg $N/m^2 \cdot$year extrapolated here is difficult to evaluate until more information is available on the nitrogen budgets of the Beaufort Sea and Eskimo Lakes regions.

7.1.3. Stimulation by Carbon Compounds

The nitrogenase activity of sediment was very greatly stimulated by the addition of glucose, sucrose, lactose, mannitol and malate. Acetate showed much less stimulation and the chitin monomer, N-acetylglucosamine showed no or negligible effect. Release of NH_4^+-N during the degradation of the N-acetylglucosamine may have been sufficient to prevent derepression of nitrogenase synthesis in the cells of the potentially N_2-fixing bacteria. In view of the possible presence of this compound in the plankton rain further study would appear desirable. Maruyama *et al.* (1974) showed that glucose, sucrose, mannitol and pyruvate stimulated acetylene reduction, but only about 14- to 30-fold, in sediments of Tokyo and Sagami Bays. Sediments of Aburatsubo Inlet were stimulated a maximum of 3-fold by the same compounds. Thus in the arctic sediments of the present study, the potential activities are very high and it would seem that the supply of carbon and energy is the major limiting factor.

TABLE 12. Reported rates of nitrogen fixation (measured by acetylene reduction assay) in sediments.

Authors	System	nmoles C_2H_4 /g·day	mg N/m^2·day
Freshwater sediments			
Howard *et al.*, 1970	Lake Erie	28 - 34	-
Keirn & Brezonik, 1971	Florida & Guatemala lakes	82 - 334	-
Sugahara *et al.*, 1971	Japan, 0-1 cm	-	3,328
Macgregor *et al.*, 1973	Wisconsin, 0-10 cm	0.08 - 1.45	-
Toetz, 1973	Oklahoma reservoir	24	-
Marine sediments			
Brooks *et al.*, 1971	Florida estuary	1.4 - 13	443
Bunt *et al.*, 1971	Card Sound, Florida	-	1 - 316
Patriquin & Knowles, 1972	Barbados	0.3 - 1.37	500 - 1,000
	New Brunswick	2.3 - 2.93	
Maruyama *et al.*, 1974	Tokyo Bay, 3°C	0.1 - 1.1	-
	20°C	1.45	-
Present report	Beaufort Sea etc. 5°C	0.1 - 0.3	25
	15°C	0.1 - 1.0	-

7.1.4. Effect of Combined Nitrogen

It is conceivable that inorganic nitrogen levels in the untreated sediments could also be a factor limiting activity by causing repression of nitrogenase synthesis. Although appropriate analyses of the samples studied were not carried out, it is interesting to refer to some analyses reported by Wacasey for Beaufort Sea stations 544, 545, 546, 548, 550, 551, 554, 555, 556, 557, 558 and 559 amongst others (Wacasey, personal communication, 1975). In these grab samples concentration ranges in µg N/g were as follows: NO_3^--N 0.58 - 1.09, NH_4^+-N 2.34 - 25.6.

Equivalent data for Eskimo Lakes stations 508, 510, 515, 520 and 523 (Wacasey, 1974) were as follows: NO_3^--N 1.2 - 4.2, NH_4^+-N 30 - 70. Repression-redepression thresholds for nitrogenase activity were reported to be less than 1 µg NH_4^+-N/g in a Barbados carbonate sand (Patriquin and Knowles, 1975) and about 5 µg NH_4^+-N/g in a soil system (Knowles and Denike, 1974). Thus the NH_4^+-N concentrations reported by Wacasey in these arctic sediments could well cause complete repression of nitrogenase activity. It is clear that this could be a subject for confirmation and further study.

7.1.5. Effect of Petroleum Products

The presence of petroleum frequently provokes an enrichment of the microbial population (Atlas and Bartha, 1972; Jobson *et al.*, 1974; Zobell, 1973) the enrichment being qualitatively and quantitatively different at low and at high temperatures (Westlake *et al.*, 1974). Hydrocarbon-utilizing bacteria were frequent in NW Atlantic waters and coastal sediments (Mulkins-Phillips and Stewart, 1974). Bunch (1975) reports that enrichment of hydrocarbon-degrading microbial populations were consistently obtained from water column samples from the Beaufort Sea and Eskimo Lakes but that they could never be obtained from sediment samples of the same areas. This finding is consistent with the present observation that neither crude oil nor the fractions used caused significant increases in CO_2 evolution or in nitrogenase activity. Indeed, the reverse was observed, in that 1,2,4-trimethylbenzene caused some inhibition of CO_2 evolution and almost complete inhibition of C_2H_4 production. It is not known to what extent this compound is a component of Norman Wells crude oil but in any case the crude oil itself showed no inhibitory effect on either nitrogenase activity or general metabolism as evidenced by production of CO_2.

Degradation of crude petroleum is frequently limited by the availability of elements such as phosphorus and nitrogen (e.g. Atlas and Bartha, 1972) but the specific enrichment of nitrogen-fixing bacteria, though presumably favoured selectively, has not been reported. However, natural gas was reported to bring about the enrichment of nitrogen-fixing methane-oxidizing bacteria (Adamse *et al.*, 1972; De Bont and Mulder, 1974; Coty, 1967) and, from soils and a refinery filter, Rivière *et al.* (1974) isolated nitrogen-fixing bacteria which utilized hexadecane as sole source of carbon and energy.

7.2. DENITRIFICATION

7.2.1. Occurrence

In an earlier study, Patriquin and Knowles (1974) showed that Eskimo Lakes sediment contained as many as 10^5 denitrifying bacteria per gram. It was not possible, in the present study,

to detect *in situ* denitrification rates (using untreated samples). However, addition of glucose and nitrate showed that a significant potential for denitrification existed. The rates of production of N_2O range up to a maximum of 53 µg N_2O-N/g·day and are thus of the same order as those reported by Garcia (1973, 1974) for many rice paddy soils in Sénégal.

7.2.2. Effect of Petroleum Products

On the basis of the rather limited data it appears that the denitrification process was not affected by weathered Norman Wells crude oil, dodecane or hexadecane, Hexane and 1,2,4-trimethylbenzene gave partial and complete inhibition, respectively, of N_2O production. There are no published reports on interactions between petroleum products and denitrification.

8. CONCLUSIONS

Very low rates of nitrogen fixation, of the order of 25 mg N/m^2·year, were detected in untreated sediments. Using the sensitive acetylene reduction assay the rates were close to the limits of detection, but were markedly stimulated by addition of glucose, sucrose and some other carbon compounds. Anaerobic and facultatively anaerobic bacteria were probably responsible for the observed activities. Studies of nitrogen fixation potentials in glucose-supplemented sediment samples showed large variation between stations, between samples from the same station and between depths within a single core down to about 12 - 18 cm. Replicability and reproducibility in experiments was therefore poor.

Denitrification was not detectable in untreated sediments but was potentially very active when sediments were supplemented with organic carbon and nitrate. Reduction of the nitrate followed the sequence $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$.

Weathered Norman Wells crude oil, decane, dodecane, and hexadecane had no effect, stimulatory or inhibitory, on nitrogen fixation, denitrification or carbon dioxide production. Hexane caused partial inhibition of denitrification, and 1,2,4-trimethylbenzene caused complete inhibition of both nitrogen fixation and denitrification but only partial inhibition of CO_2 production. There was no evidence of utilization of any of the hydrocarbons during periods of over 30 days.

9. IMPLICATIONS

9.1. Scientific

Nitrogen fixation and denitrification activities were low and undetectable, respectively, in untreated sediments but were potentially high when appropriate substrates were made available. There was no short-term (30-40 days) effect of crude oil or of certain petroleum fractions on either process. Tri-

methylbenzene inhibited both nitrogen fixation and denitrification but the mechanism of this inhibition was not investigated.

9.2. Offshore Drilling

Data suggest that moderate crude oil contamination would not affect nitrogen fixation and denitrification in the sediments.

9.3. Views

Although no consistent effects of crude oil on the processes studied were observed in periods of up to 30-40 days it is possible that a long term enrichment of hydrocarbon-utilizers and even of nitrogen-fixers might occur. It seems rather unlikely that significant inhibition would develop on a long term basis.

10. NEEDS FOR FURTHER STUDY

Methods by which homogeneity of sediment could be increased for experimental work should be studied. Further attempts to investigate the *in situ* rates of nitrogen fixation and to detect *in situ* denitrification need to be made. Analyses of sediment, particularly for inorganic forms of combined nitrogen, should be confirmed and extended. The possibility of long-term enrichment of hydrocarbon-utilizers, nitrogen fixers or denitrifiers should be studied over periods of several months at low temperatures.

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13. APPENDIX TABLES 1, 2 and 3

Appendix Table 1

Beaufort Sea Samples

Station No.	Date	Cores	Grab samples
7	July 22-30, 1974	1	
9	July 22-30, 1974	1	
12	July 22-30, 1974	1	
14	July 22-30, 1974	4	
22	Aug. 12, 1974	2	1
23	Aug. 15-16, 1974	1	1
29	Aug. 2, 1974	2	1
30	Aug. 2, 1974	2	1
554	During cruise in	1	1
545	Aug.-Sept., 1974	1	1
546			1
547		1	1
548		1	1
549		1	1
550		1	1
551		1	1
552		1	1
553		1	1
554		2	1
555		1	1
556		2	1
557		1	1
558		1	1
559		1	1
566	July 15, 1975		1
568	July 19, 1975		1
565	July 25, 1975		1

Appendix Table 2

Eskimo Lakes Samples

Station No.	Date	Cores	Grab samples
508	Feb. 27, 1974		4
	May 24		2
	July 27	1	1
	Aug. 3	2	
	Aug. 15	1	1
	Aug. 21	4	1
510	Aug. 8	1	1
515	July 22		1
	July 27	2	1
	July 30		1
	Aug. 3	2	1
	Aug. 15-19		1
	Aug. 19	4	1
520	Aug. 7	1	1
523	Aug. 7	1	1
550*	Aug. 8	1	1
515	July 15, 1975		1
	July 26, 1975		1

*Clupea Harbour, Eskimo Lakes

Appendix

Table 3. Production of C_2H_4 (nmoles/g) and CO_2 (μmoles/g) during 132 hours of incubation of glucose-amended (0.5% w/w) sediment from various stations in the Beaufort Sea and Eskimo Lakes.

Station No.	pH*	C_2H_4	CO_2
Beaufort Sea:			
22	7.1	64	2.6
23	7.1	82	3.2
29	7.4	51	3.2
30	7.5	32	1.8
544	7.5	139	6.2
545	7.1	467	10.7
546	7.4	131	2.9
548	7.3	133	3.2
551	7.6	28	1.9
554	7.5	9	2.0
555	7.6	36	3.0
556	7.4	302	5.3
557	7.4	230	4.1
558	7.2	129	3.0
559	7.3	149	3.2
Eskimo Lakes:			
510	7.5	208	3.4
515	7.3	331	5.5
520	7.2	357	3.5
523	7.4	476	5.8
550	7.3	350	6.0

* pH values were measured on suspensions (dry weight: distilled water ratio, 1:4) after "Vortex" mixing and one hour of equilibration.