Methane Production and Release from a Northern Wisconsin Peat Bog

BIOS 569 - Practicum in Aquatic Biology

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I. Abstract

The production and release of methane was studied in a northern peat bog in Wisconsin through the use of three different experimental methods. Rates of methane release at the surface were determined with the use of methane release chambers. The distribution of methane in the sediments was determined with the use of interstitial dialysis sticks, and in-vitro experiments were done with additions of sodium sulfate and sulfuric acid to determine the effects of sulfate, at varying pH's, on methanogenesis.

Two separate experiments were done with the methane release chambers. The first experiment involved sampling over a 28 hour period. The average rate of methane release over the 28 hour period was -10.69 umol/m^2 hr. Samples taken on an hourly basis showed rates as high as +.46 mmol/m^2 hr. The second methane release chamber experiment involved sampling over a 66 hour period. The average rate of methane release over the 66 hour period was +5.94 umol/m^2 hr.

Methane concentrations increased rapidly in the sediment directly below the water table, and then steadily increased with increasing depth down to about 12 cm below the water table. Methane concentrations at depths greater than 12 cm below the water table showed a slight decline.

Neither sodium sulfate nor sulfuric acid affected the production of methane in the in-vitro experiments.

Methane Production and Release from a Northern Peat Bog

II. Introduction

The 1% increase in atmospheric methane concentration per year (Lansdown et. al., 1992) is definitely reason for concern, considering that a methane molecule has a greenhouse effect 37 times that of a carbon dioxide molecule. Currently, the atmospheric methane concentration is about 1.72 ppmv., and wetlands, the largest natural source of methane, contribute about 20% of the global methane source (Lansdown et. al., 1992). Bogs are a type of wetland often dominated by sphagnum moss. Sphagnum releases H+ ions, in exchange for important cations, and thus promotes an acidic environment (Wetzel, 1983) It is this acidity, and the saturation of the bog soil with water, that causes the slow decomposition rates, and the resulting accumulation of organic material (Yavitt et.al., 1987).

Because bog soil is saturated with water, oxygen diffuses down into it very slowly. The diffusion is so slow that there is not enough oxygen present in the soil below the water table to support aerobic organisms. Thus the soil is home to many anaerobic bacteria. Both sulfate-reducing and methane-producing bacteria play important roles in anoxic sediments (Winfrey and Ward, 1983). Methane production in anoxic environments can occur in two ways (Lansdown et.al., 1992). Both are microbially mediated reactions, but there are different substrates involved. Aceticlastic methanogenesis takes acetate and hydrogen gas to methane and carbon dioxide. Carbon dioxide reduction takes carbon dioxide and hydrogen gas to methane and water (Lansdown et. al. 1992).

Although the subject of this paper is methane production, sulfate-reducing bacteria are important to mention, because of their ability to outcompete the methane-producing bacteria of aceticlastic methanogenesis for acetate and hydrogen gas. Because sulfate-reducing bacteria are better at consuming acetate and hydrogen gas than methane-producing bacteria, they are able to inhibit methanogenesis in the presence of sulfate (Winfrey and Ward, 1982).

Methylotrophs affect the overall release of methane from sediment surfaces (Fechner and Hemmond, 1992).

Methylotrophs are methane-oxidizing bacteria that oxidize methane to carbon dioxide in the presence of oxygen (Fechner and Hemond, 1992). Thus, the amount of methane released from the surface of a bog is not always indicative of how much methanogenesis or carbon dioxide reduction is going on deep within the sediments. The methane produced within the saturated sediments can be oxidized on its way to the atmosphere when it passes through the upper oxic unsaturated zone of peatland (Fechner and Hemond, 1992). The oxidation of methane to carbon dioxide has also been observed in anoxic environments at the sediment surface (Wetzel, 1983). It is not clear how this occurs, but it seems to involve the activity of sulfate-reducing bacteria (Wetzel, 1983).

III. Project Description

A. Topic Details

Methane production can occur in anoxic conditions via two pathways. Aceticlastic methanogenesis and carbon dioxide reduction are both biological activities that result in the production of methane. Aceticlastic methanogenesis can be inhibited in the presence of sulfate by sulfate-reducing bacteria.

Once the methane is produced under anoxic conditions, it can be oxidized to carbon dioxide by methylotrophs, if it passes through an oxic zone.

B. Hypotheses

Two hypotheses were being tested in this study. The first one predicted an increase in methane production with an increase in depth. The reasoning is that as one moves further away from the atmosphere into the waterlogged soils, and the concentration of oxygen in the soils decreases, anaerobe concentrations will increase. Higher concentrations of anaerobes should lead to an increase in anaerobic activity. Because methane production is an anaerobic activity, one would expect it to be higher at greater depths, where less oxygen is available. The second hypothesis predicted a decrease in methane production with an increase in sulfate. In the presence of sulfate, sulfate-reducing bacteria are expected to outcompete methane-producing bacteria for acetate and hydrogen, the substrates necessary for

aceticlastic methanogenesis.

IV. Materials and Methods

A. Study Site

The bog that was studied in this experiment is called Donut Bog. It is a sphagnum peat bog located on the University of Notre Dame Environmental Research Center property in northern Wisconsin. Donut Bog is approximately 80 yards long and 50 yards wide. It is dominated by green sphagnum, and varies in water content depending on the season. At the time of this study, the water table was at 10 cm. below the surface at the north and south ends of the bog, and was at 17 cm. below the surface in the central portion of the bog. The bog is acidic, with a surface Ph of 3.77.

B. Gas Chromatography

Methane concentrations were determined with a Hewlett Packard 5890 Series II gas chromatograph.

C. Sampling Procedure

The first method of sample collection involved the placing of circular methane release chambers (Fig.1) down over the surface of the sphagnum. All of the chambers were plugged in the center with a septum, allowing gas samples to be drawn from them with a needle into 10 ml. vacutainers. The samples were equilibrated by shaking the vacutainers. Each sample was then run through the gas chromatograph.

Fechner and Hemond (1992) showed that approaching a flux chamber by foot increased the methane flux by a factor of ten. In this study the release chamber measurements were made by approaching the chambers by foot. Special care was taken to disturb the sediments as little as possible, but disturbance did occur and is thus a source of error. Another source of error stems from the problem of creating a tight seal between the sphagnum and the methane release chambers. Cinder bricks were placed on top of the release chambers in an attempt to create a tight seal, but gas was probably able to diffuse out from the bottom sides of the pan.

Samples were also collected using interstitial dialysis samplers (Fig.1). These plexiglass samplers

have 12 chambers, spaced three cm. apart in a vertical column. The chambers in the dialysis samplers were covered with a membrane, which was kept in place by another piece of plexiglass that fit over the top of the samplers, but did not cover the chambers. The samplers were bubbled with nitrogen for about 18 hours in a large plastic graduated cylinder. Foil was placed over the top during the bubbling to avoid any contamination with air. The samplers were taken into the field, immediately following the bubbling, in the large graduated cylinder. They were then pushed into the bog sediments so that the third chamber of each sampler was at the water table. The samplers were removed from the sediments three days later. Upon the removal of each sampler, a needle was used to draw a 10 ml. sample from each chamber into a labeled 10 ml. vacutainer. The samples were all shaken and then layed out horizontally for six hours so that equilibration could occur. The samples were periodically shaken throughout the six hour period. Gas samples from the headspaces of the vacutainers were then injected into the gas chromatograph.

This method of sampling also disturbed the sediments, both in the insertion and removal of the samplers, and may have caused artificially induced changes in the methane gradient in the sediment, which would be reflected in the samplers, and thus in the samples.

A core sampler was used for sampling the bog sediments used in the in-vitro experiments. Glass jars were first deoxygenated with nitrogen, sealed, and then taken into the field. The core sampler was used to make a large hole down into the sediment, so that sediment samples at desired depths could be placed into the glass holding jars. The samples were then rushed to the lab at ambient temperature, where small portions of each sample were placed into 30 ml. vials. The vials were deoxygenated with nitrogen and then sealed. Treatments were added to the vials with a syringe and needle, through the septum at the top of each vial. After a certain incubation time, samples from each vial's headspace were injected into the gas chromatograph.

The main source of error with this sampling

method is the extensive amount of handling that occurred with the sediment. It was handled both in the field and in the lab, and was inevitably exposed to oxygen for short periods of time. This exposure might have allowed for some biological oxidation of methane, before the incubation. Kelly and Chynoweth (1980) showed that in freshwater sediments, surface sediments could be preserved for in-vitro studies, but that deeper sediments produced erroneous results.

D. Nutrient Additions

Three treatments were used in the in-vitro experiments. All three treatments were bubbled with nitrogen before being injected into the vials with a 10cc syringe and needle. The three treatments were distilled water, sodium sulfate, and sulfuric acid. Distilled water served as the control. The sodium sulfate was at a Ph of 7.02, and the sulfuric acid, which was meant to simulate acid rain, was at a Ph of 3.83.

V. Results

The first methane release chamber experiment was done at sites A and B (Fig.2). There were two repetitions per site, and thus a total of four release chambers. Samples were taken at time 0 (t0) and then periodically over the course of 28 hours. Although hourly samples sometimes showed flux rates as high as +.46 mmol/m^2 hr., the average flux rate over the 28 hour period was -10.69 umol/m^2 hr. The second release chamber experiment was done at sites A, B, C, and D (Fig.2). Again there were two repetitions per site, for a total of eight release chambers. Samples were taken periodically over a 66 hour time period, showing an average flux rate of +5.94 umol/m^2 hr.

The interstitial dialysis sampler experiment was done at sites A, C and D. Samplers two and eight were at site A, seven and one at site C and three and four at site D (Fig.2). The third chamber of each sampler was placed right at the water table, with chambers one and two above the water table, and chambers four to twelve below. Because the chambers on a sampler

are three cm. apart, the twelth chamber on each sampler was 27 cm. below the water table. In general, uM concentrations of methane were low in all of the samplers from depths of +6 cm. above the water table to -3 cm. below the water table. Concentrations increased dramatically from -3 cm. to -6 cm below the water table, and then continued to increase with increasing depth. At about -15 cm., concentrations fell slightly, but were still much higher than concentrations in the zone near the water table. The results in the zones -15 to -27 cm. below the water table were much more ambiguous than the results for the sediments above (Fig.3).

The purpose of the first in-vitro experiment was to see how well the gradient observed in-vitro matched the gradient observed in the field. The treatment of 10cc distilled water was added to 12 vials, two from each depth. Depths were in cm., with 0 being the depth at the water table. Depth increments were 0-10, 10-20, 20-30, 30-40, 40-50, and 50-60. Incubation time was seven days. A shorter incubation time had been planned, but problems with the gas chromatograph delayed the quantification of the samples. Methane concentrations were the lowest in the sediments found 0-10 cm. below the water table, and 40-60 cm. below the water table. Concentrations were the highest in the sediments from 20-30 cm. below the water table (Fig.4).

The second in-vitro experiment used sediments from the same six depths as in the first. There were three treatments for each depth increment, with two replicates per treatment, for a total of six samples for each depth increment. The three treatments were distilled water, sodium sulfate, and sulfuric acid. Each sample was run on the gas chromatograph three separate times. The first run was after twelve hours (Fig.5), the second was after forty-eight hours (Fig.6), and the third was after seventy-two hours (Fig.7). There were no definite trends with changes in depth. Also, the treatments of sulfate definitely did not inhibit the production of methane. In fact, it seems possible that the additions of sulfate actually promoted methane production.

VI. Discussion

The results from the methane-release chambers suggest that methane was probably able to diffuse out of the chambers, causing the methane concentration to stay about the same in the chambers over the sampling period. The negative average flux in the first experiment suggests that this was occurring, as well as the fact that changes in flux rate on an hourly scale were much higher than the changes in flux rate over the entire sampling period.

The hypothesis that predicted an increase in the concentration of methane with an increase in depth was tested by the interstitial dialysis sampler experiment. The results from this experiment support the hypothesis down to about 15 or 18 cm., but below this depth the picture is much less clear. Methane concentrations seem to peak at about 15 cm. below the water table, and then drop off as depth increases further. Fechner and Hemond (1992) studied the gradient of methane concentration in a sphagnum peatland, and found that methane concentrations did increase with increasing depth, but they only went down to the depth of the water table. They also observed greater heterogeneity of methane concentrations in the sediments near the water table than in the sediments above, which is supported by the results in this experiment, which show greater heterogeneity in the sediments both near the water table and below.

It was predicted that concentrations of methane would increase with increasing depth, because of the fact that methane production only occurs in anoxic environments, and the environment in sediments is more anoxic at deeper depths. The results of this experiment suggest that other factors might be involved as well. Winfrey and Ward (1983) found that methane production in intertidal sediments was greatest at the surface and decreased with sediment depth. Yavitt, Lang, and Wieder (1987) studied peat from a sphagnum bog called Big Run Bog in West Virginia, and also found decreased methane production with an increase in depth. Their studies, however, were of the sediments in-vitro, and as mentioned

before, Kelly and Chynoweth (1980) were unable to preserve deeper sediments for in-vitro experiments. Methane production is dependent upon the presence of specific substrates, so one possibility for the decrease in methane production at lower depths is that of substrate limitation. Yavitt et al (1987), after doing experiments with glucose concluded that sediments below the 15 cm. depth were limited by the availability of substrates derived from glucose degradation. Additions of acetate increased production of methane only in depths lower than 25 cm., and only when it was accompanied by a pH adjustment to 6.4. Yavitt et al., then tested for hydrogen limitation, which is a possible glucose degradation product, and found that H_2/CO_2 amendments stimulated methane production in sediments deeper than 20 cm. This suggests, that in Big Run Bog the pathway for methane production in deeper sediments is through CO_2-reduction, which is limited by H_2. Methane production in the deeper sediments of Donut Bog might also occur through the CO_2 reduction pathway, with H_2 as the limiting factor. Methane production can also be limited by substrates used in methanogenesis, such as acetate. Winfrey and Ward (1982) found that methanogens in intertidal sediments may use methylamine as their substrate.

The first in-vitro experiment was designed to test the accuracy of in-vitro experiments compared to what actually occurs in the field. The results do not support Kelly and Chynoweth (1980). The gradient of methane production observed in-vitro was similar to what was observed in the field, and indicates that the sediment was able to be preserved. The second in-vitro experiment tested the hypothesis that additions of sulfate would inhibit the production of methane. Additions of sulfuric acid or sodium sulfate did not inhibit the production of methane, and thus it appears that either sulfate-reducers were absent from the sediment, or that they were competing for different substrates than the methane producers. As mentioned before, Yavitt et. al. found that methane production was most likely occurring along the CO_2 reduction pathway in

deeper sediments. Also, Lansdown et. al. found that CO_2 reduction could account for all the methane production in a temperate bog. So it seems likely that Donut Bog is dominated by methane production through the CO_2-reduction pathway, such that methane producers are not competing with sulfate-reducers for acetate, but rather are limited by the absence of H_2. Yavitt et. al. also found that CH_4 production was stimulated in deep peat by the addition of sulfate. One possible explanation for this is that somehow the addition of sulfate to the deep peat started a cascade of anaerobic reactions which led to the release of substrates such as hydrogen, which would in turn increase methane production.

VII. Acknowledgements

I am very grateful to The Bernard J. Hank Family Endowment for this research opportunity. I would also like to thank my adviser Dr. R.G. Carlton for his help and advice, my UNDERC classmates for their help and support, Mark Lavery, Rich Huftalen, Jean Keaveney, Dr. Marty Berg, and all the UNDERC faculty, graduate students and lab technicians.

VIII. References

Fechner, E.J. and H. Hemond. 1992. Methane Transport and Oxidation in the Unsaturated Zone of a Sphagnum Peatland. Global Biogeochemical Cycles, 6(1),33-34.

Kelly, C.A. and D.P. Chynoweth. 1980. Comparison of In Situ and In Vitro Rates of Methane Release in Freshwater Sediments. Applied and Environmental Microbiology, August: 287-293.

Lansdown, J.M., P.D. Quay, and S.L. King. 1992. CH_4 Production Via CO_2 Reduction in a Temperate Bog: A Source of 13C-depleted CH_4. Geochimica et Cosmochimica Acta, 56: 3493-3503.

Wetzel, R.G. 1983. Limnology. Second Edition. Philadelphia, W.B. Saunders Co., pp. 601, 743.

Winfrey, M.R. and D.M. Ward. 1983. Substrates for Sulfate Reduction and Methane Production in Intertidal Sediments. Applied and Environmental Microbiology, January: 193-199.

Yavitt, J.B., G.E. Lang, and R.K. Wieder. 1987. Control of Carbon Mineralization to CH_4 and CO_2 in Anaerobic, Sphagnum-derived Peat from Big Run Bog, West Virginia. Biogeochemistry, 4: 141-157.

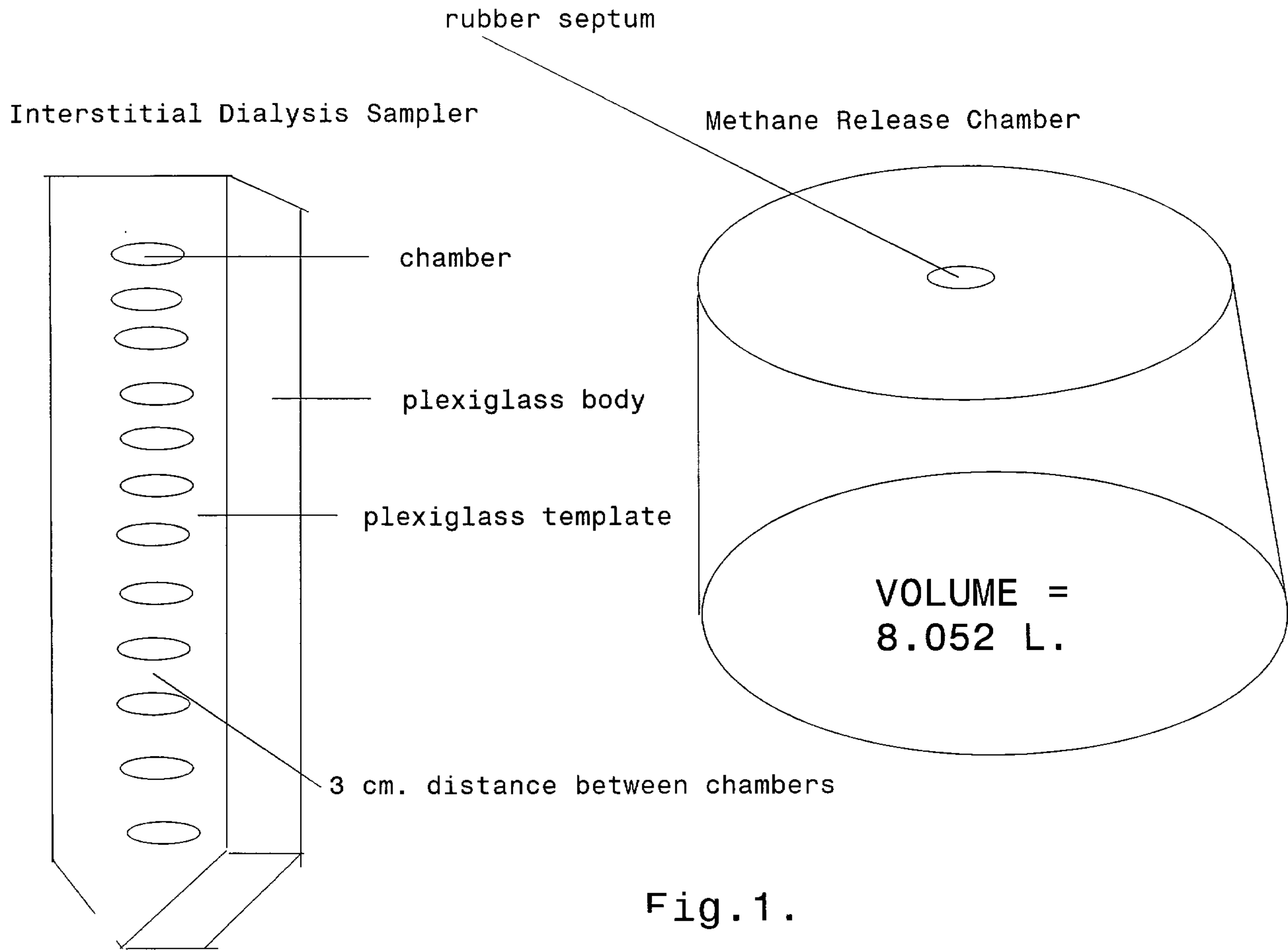
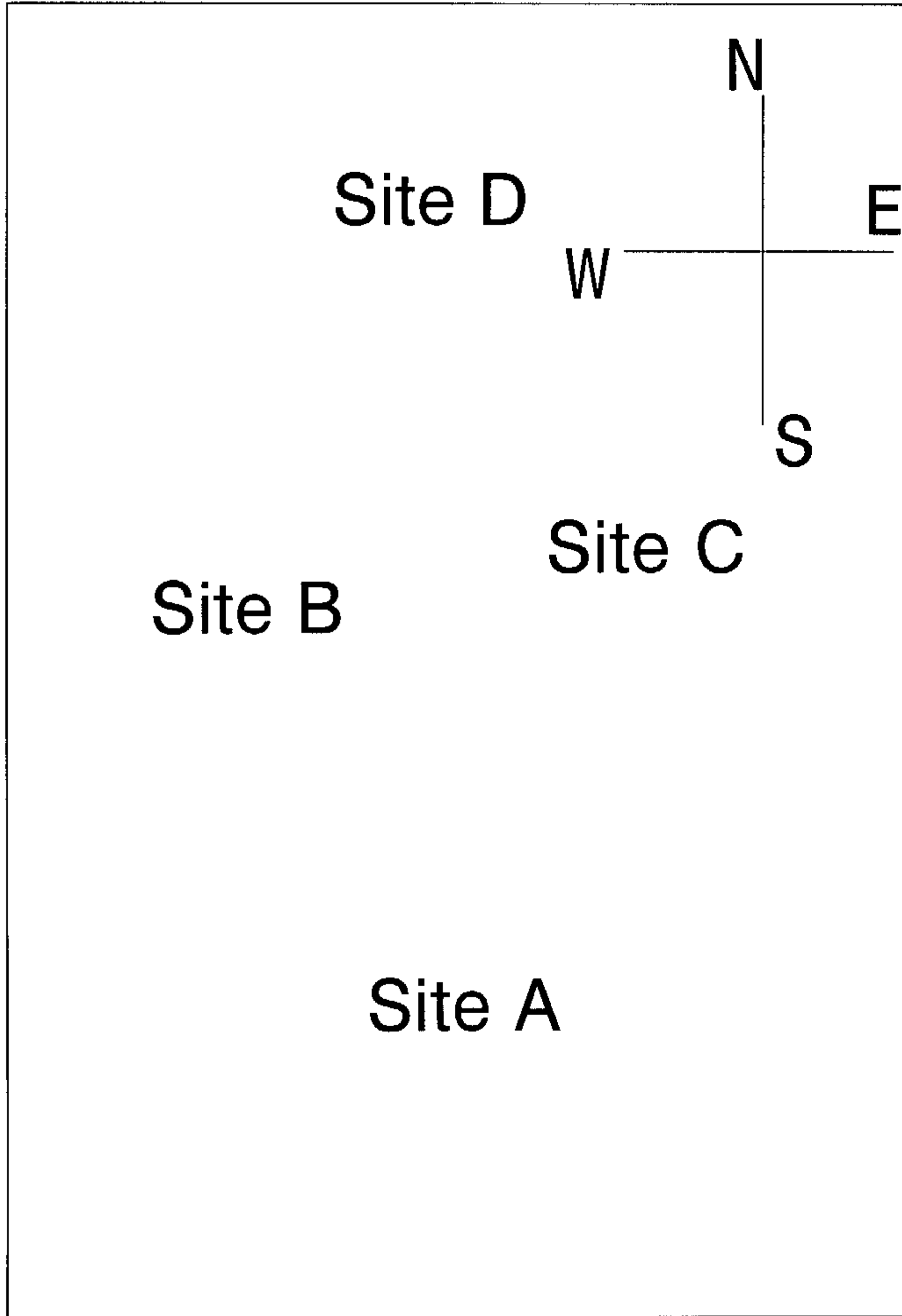


Fig.1.

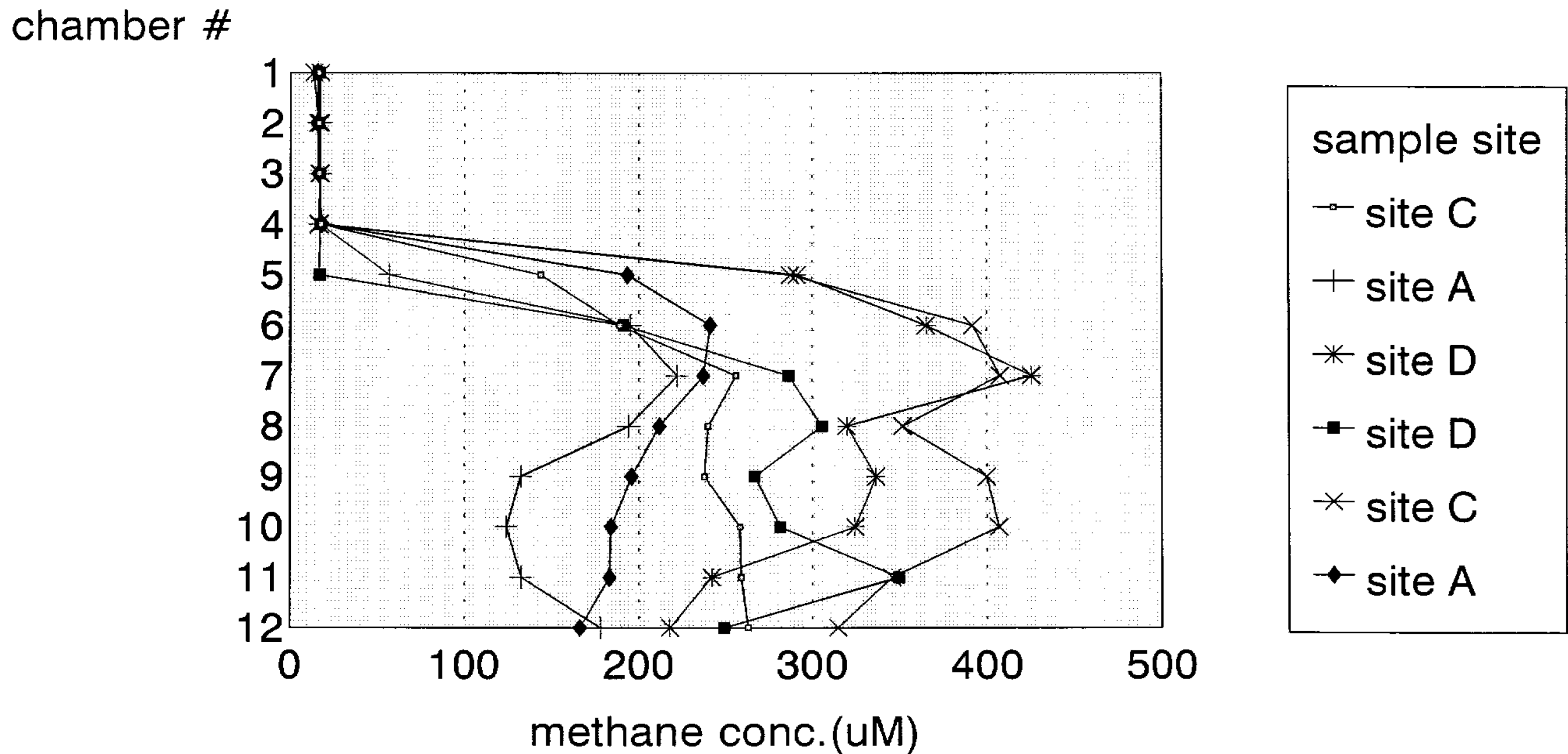
Fig.2.
Sampling Sites at Donut Bog



Concentrations of Methane along a Depth Gradient

Samples Taken from Donut Bog

Fig.3



chamber #1 of each sampler is located at bog surface
chamber #3 of each sampler is located at phreatic surface

In-vitro Methane Release by Sediments of Increasing Depth

Sediments taken from Donut Bog

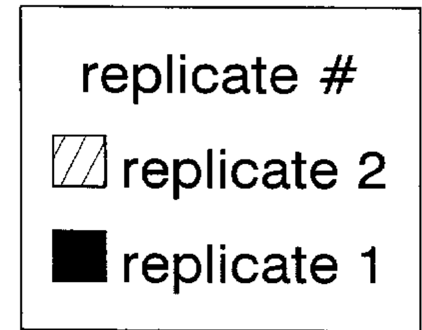
Fig.4

thousands (umol methane/g peat)

10
8
6
4
2
0

0 10 20 30 40 50 60 70

depth (cm.)



depth = cm. below water table

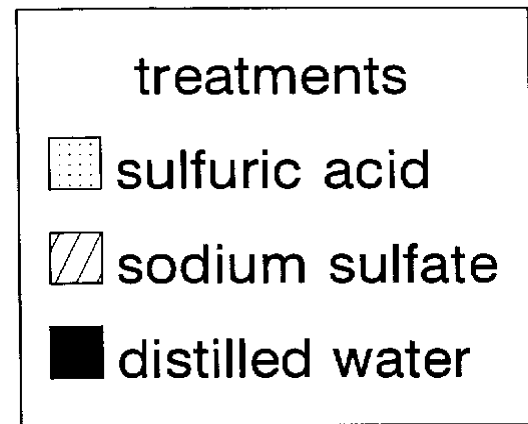
incubation time = seven days

In-vitro Methane Production with Manipulation of Depth and Treatment
Fig.5

umol CH4/g peat

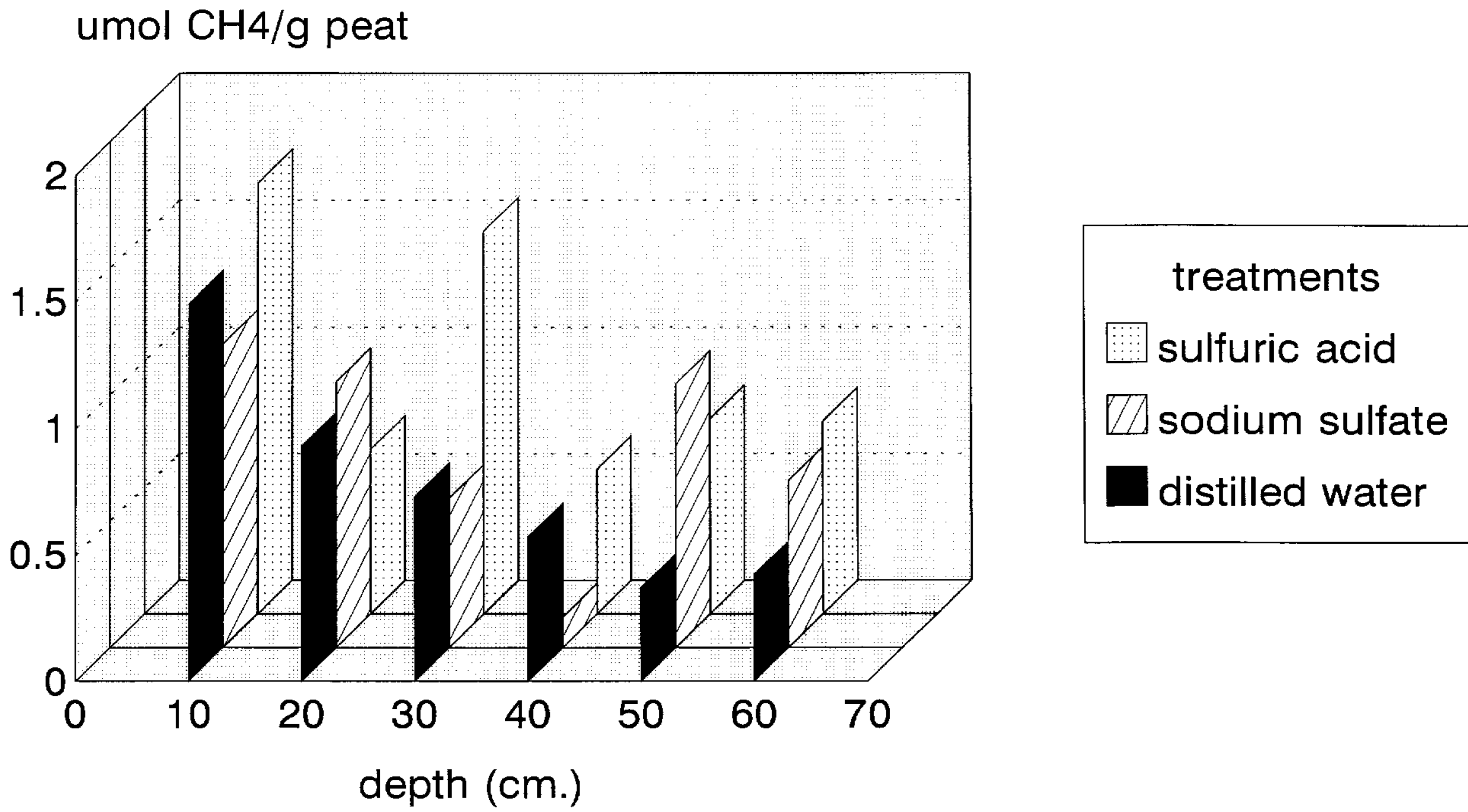
1
0.8
0.6
0.4
0.2
0
0 10 20 30 40 50 60 70

depth (cm.)



depth = cm. below water table
samples were run 24 hours after treatment

In-vitro Methane Production with Manipulation of Depth and Treatment
Fig.6



depth = cm. below water table
samp'-s were run 48 hours after treatr-ent

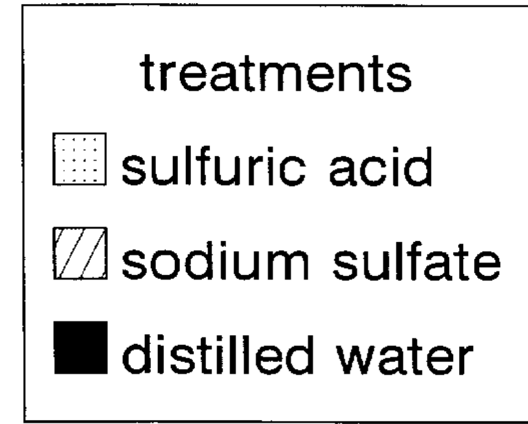
In-vitro Methane Production with Manipulation of Depth and Treatment
Fig.7

umol CH4/g peat

3.5
3
2.5
2
1.5
1
0.5
0

0 10 20 30 40 50 60 70

depth (cm.)



depth = cm. below water table
samp'-s were run 72 hours after treatm-ent

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