Reports

Transformation of a Tundra River from Heterotrophy to Autotrophy by Addition of Phosphorus

Abstract. *Continuous enrichment of an arctic river with only 10 parts per billion phosphate-phosphorus caused an immediate growth of attached algae for more than 10 kilometers downstream, showing that phosphorus alone limited photosynthesis. As a result of the increased photosynthesis, there was an increase in bacterial activity in films on rocks on the bottom of the stream. The major source of energy became the photosynthetic carbon fixed in the stream rather than the organic material entering from the surrounding tundra, and the overall metabolism of the stream shifted from heterotrophy to autotrophy. An increase in the size and developmental stage of some of the dominant aquatic insects illustrates the food limitation in this nutrient-poor habitat.*

BRUCE J. PETERSON
JOHN E. HOBBIE
Marine Biological Laboratory,
Woods Hole, Massachusetts 02543
ANNE E. HERSHEY
University of Wisconsin,
Madison
MAURICE A. LOCK
TIM E. FORD
University College of North Wales,
Bangor, United Kingdom
J. ROBIE VESTAL
VICKY L. MCKINLEY
MEREDITH A. J. HULLAR
MICHAEL C. MILLER
University of Cincinnati,
Cincinnati, Ohio
ROY M. VENTULLO
GREGG S. VOLK
University of Dayton,
Dayton, Ohio

The activities of man in virtually any landscape result in disturbance of the elemental cycles and nutrient enrichment of downstream ecosystems (*1*). The consequences of this enrichment are well known for lakes but are less well known for rivers (*2*), in part because many temperate rivers are already seriously affected by human activities (*3*). To study the effect of enrichment, we have investigated an arctic stream essentially unaffected by man's activities. This stream was enriched by the continuous addition of dissolved phosphorus for 6 weeks.

Phosphoric acid was added to the Kuparuk River, a meandering tundra stream in northern Alaska (*4*), during July and August 1983. The dripping rate from a Mariotte bottle was adjusted daily so that the concentration of dissolved phosphate in the stream was increased by 10 μg phosphate-phosphorus (PO_4-P) per liter (this did not measurably change the *p*H of the stream water). The river immediately upstream from the addition site served as a control. After 26 days of enrichment (1 August), we measured microbial processes in the riffles and pools and collected insects for study. For additional experiments on light and nutrient effects, transparent and opaque flow-through plastic tubes submerged in the river were used (*5*). Stream discharge ranged from 0.5 to 5 m^3 per second, and water temperature was between 7° and 14°C most of the time.

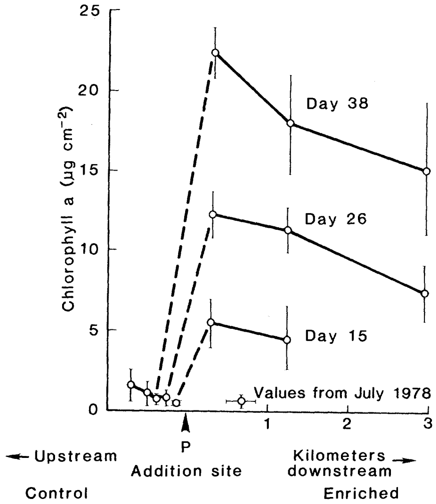

Fig. 1. Concentration of chlorophyll a on stream bottom rocks in riffles upstream and downstream of the point where phosphorus was continuously added to the stream. Data represent means ± 1 standard deviation with *n* = 16 (1978) and *n* = 4 or 5 (1983) at each sampling site.

The strong response of algal growth to the added phosphorus confirms that photosynthesis of algae in the stream was limited by this single element. The biomass of the epilithic algae (*6*), as measured by chlorophyll concentration (*7*), soon increased by a factor of 10 (Fig. 1). This effect extended for many kilometers downstream; we found 0.5 μg of chlorophyll a per square centimeter in the upstream control section and 6 μg/cm^2 10 km below the enrichment point. Chlorophyll suspended in the river water also increased (1 to 2 μg/liter upstream compared to 30 μg/liter downstream) because of sloughing of algae from the epilithon. Another indicator of epilithic production was the organic carbon content of the river water that increased in the 0.7 μm to 300,000 apparent molecular weight fraction from 0.21 mg of carbon per liter in the control section to 0.49 mg/liter at 300 m below the enrichment site (*8*).

After 6 weeks of enrichment, the attached diatom assemblage at sites immediately below the point of phosphorus addition showed a reduction in density of an oligotrophic indicator species [*Tabellaria floculosa* Kutz (*9*)], a reduction in the number of species per unit area (38 cm^2), and a reduction in evenness. Still farther downstream, all community parameters increased again. Polar ordination of sites by species separated phosphorus-enriched sites from both the upstream control and 3000-m downstream site. Six species were dominant at all sites, but after addition of phosphorus some rare species were not found at stations downstream of the phosphorus addition site. The total number of diatom species found was 39 above the enrichment, 21 at 30 m below the enrichment, and 26, 29, and 33 species at 200, 1200, and 3000 m, respectively. Phosphorus addition simplified the diatom community by allowing increased dominance by common species while a number of rare species became even more scarce (*10*).

The increase in the biomass and productivity of epilithic algae led to increases in the removal of dissolved reactive phosphorus and of nitrate below the phosphorus addition point. Ambient levels of nitrogen and phosphorus in the Kuparuk range from 5 to 80 μg of NO_3-N per liter, from 2 to 30 μg of NH_4-N per liter, and from <1 to 4 μg of PO_4-P per liter. Two and three weeks after fertilization began, up to two-thirds of the ambient nitrate in river water was taken up within 4 km of the phosphorus addition point. Levels of dissolved inorganic carbon were high enough (2 to 8 mg of total CO_2-C per liter) so that carbon availability did not limit algal growth.

Most of the metabolism in the stream takes place in the microbial community attached to rocks (the epilithon); the activity of various components of this community may be sorted out in experiments with organisms grown on small glass beads colonized in either enriched or control sections of the river and under light or dark conditions. In one type of experiment, the beads were placed in a calorimeter to measure heat production (*11*) from the microbes, a measure of metabolic activity. The activity on beads colonized in the light was increased to three times as much as controls when the beads were colonized either in the enriched section of the stream (an addition of 10 μg of phosphorus per liter) or in flow-through chambers with 10 μg of phosphorus per liter plus 100 μg of NO_3-N per liter (Fig. 2A). The activity on beads colonized in the dark was not increased by added nutrients. In the upstream sections (control), the activity was only slightly higher on beads colonized in the light than on those colonized in the dark. Because light had little effect, we conclude that the metabolism of the unenriched stream is dominated by heterotrophs which obtain their energy from carbon entering the stream from the tundra. When the growth of epilithic algae is stimulated by nutrients, the algae, or the combination of algae plus associated bacteria, become the dominant organisms for carbon flow. This autotrophic metabolism completely overshadows heterotrophic growth which depends predominantly on the use of organic matter from terrestrial sources.

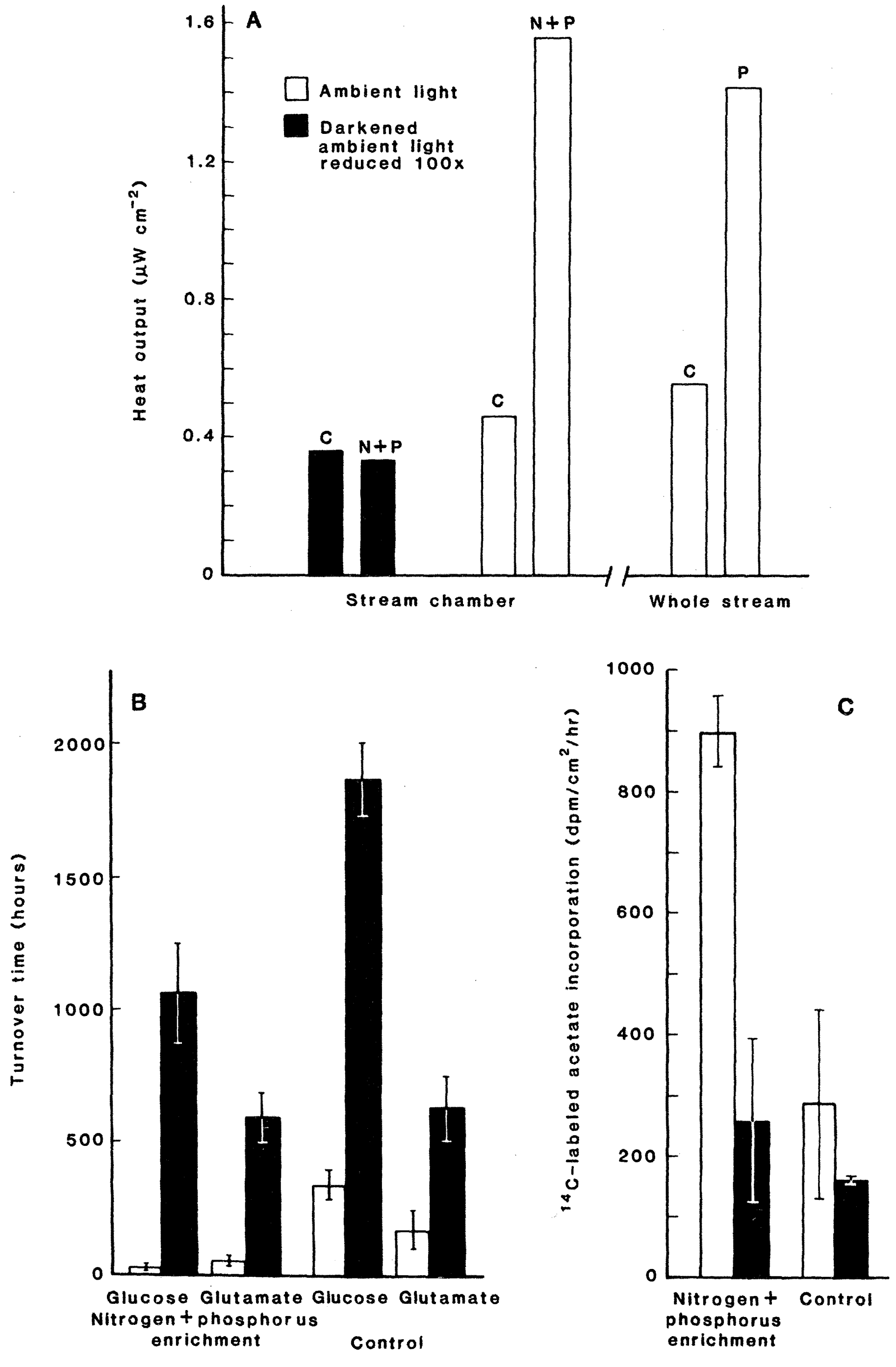

Fig. 2. (A) Metabolic heat production from respiration of the epilithon community that developed in light (white bars) and darkness (dark bars) with (P and N + P) and without (C) nutrient enrichment in the flow-through bioassay tubes and in the whole-stream enrichment. (B) Turnover (uptake and respiration) of glucose and glutamate by epilithic bacterial communities grown under light and dark conditions with and without inorganic nutrients. Samples were taken after 26 days of colonization in the continuous-flow bioassay tubes. Short turnover times indicate high activity. Data represent means ± 1 standard deviation (n = 4). (C) Microbial activity as measured by ^{14}C-labeled acetate incorporation by epilithon that developed under light and darkness with and without inorganic nutrient enrichment in the continuous-flow bioassay tubes. Data represent means ± 1 standard deviation.

When the bacterial activity alone was analyzed, it was found to be correlated with the growth of algae (Fig. 2B). The activity was measured in one way as the turnover of extremely small quantities (0.5 μg/liter) of ^{14}C-labeled glucose and glutamate added to slurries from epilithon from the beads (*12*). The highest activity (the lowest number of hours) occurred with bacteria grown in the light with added nutrients. A second measurement of bacterial activity, the incorporation rate of ^{14}C-labeled acetate into microbial lipids (*13*), gave similar results (Fig. 2C). Evidently the bacteria used the organic carbon from algal exudates and lysis to increase their growth and activity. Additions of inorganic phosphate and nitrogen in the dark stimulated heterotrophic activity only slightly.

The changes in bacterial activity were mirrored in the changes in bacterial biomass. By direct count (*14*), there were 2.6×10^6 cells per square centimeter on the beads colonized in the dark either with or without nutrient enrichment. Colonization in the light resulted in an increase to 4.2, and light plus nutrient enrichment produced 9.2×10^6 per square centimeter. The lipid phosphate content (*15*), which is proportional to the biomass of all microbes, gave values of 69 ± 11 (mean ± 1 standard deviation) and 160 ± 20 nmol/cm^2 for the dark and light controls and 92 ± 11 and 1551 ± 90 nmol/cm^2 for the beads colonized with added nutrients in the dark and in the light. The algal biomass as measured by chlorophyll a was 0.2 μg/cm^2 and 4.6 μg/cm^2 for the dark and light controls and 0.2 and 20.6 μg/cm^2 for the beads colonized with added nutrient in the dark and in the light, respectively. The algae may have contributed much of the lipid phosphate in the light plus nutrient treatment because the increases in lipid phosphate and in chlorophyll were much greater than the increase in bacterial cell numbers.

The activity of the decomposers of particulate organic matter was not changed as a result of the phosphorus enrichment if the organic matter was relatively labile. First, there were no differences in weight loss of leaves of a common sedge *(Carex aquatilis)* incubated in mesh bags in the control and enriched sections of the river. Second, the rate of incorporation of acetate into microbial lipids (*13*) was not different between *Carex* leaves from the control and enriched sections of the river. These results agree with those of Howarth and Fisher (*16*) who found no effect of nutrient addition on the decomposition of sugar-maple leaves but do not agree with results of Elwood *et al.* (*17*), who used the more recalcitrant red oak leaves in temperate woodland streams.

For this meandering arctic stream the main input of particulate carbon is the peat that erodes from the stream banks (*18*). Because this material is much more recalcitrant to decomposition than fresh, green *Carex* leaves (*18*), we also examined the effect of enrichment on the mineralization rate of refractory material. Purified *Carex* ^{14}C-labeled cellulose and lignin-lignocellulose (*19, 20*) mineralization were significantly stimulated by phosphorus addition when these resistant materials were incubated in water with an inoculum of bacteria from Kuparuk peat. We conclude that the response of the decomposer community to added nutrient may be determined by the biochemical composition of the substratum. The breakdown of refractory materials could be accelerated by added phosphorus, whereas decomposition of labile organic matter (^{14}C-labeled glucose, measured in a similar incubation with peat inoculum) was not changed by the phosphorus addition. Previous work (*21*) revealed that fresh, green *Carex* litter contained 0.64 percent phosphorus, whereas degraded, brown *Carex* litter contained 0.09 percent phosphorus.

The dominant riffle insects in the stream also responded to the nutrient addition by increasing their growth (there was not enough time for them to reproduce during the experiment). After 26 days of phosphorus addition, the larvae of the blackfly, *Prosimulium* sp., were significantly larger in the enriched section than in the control section (Fig. 3) (*22*), probably because of the increased abundance of algal cells in the water. Merritt *et al.* (*23*) found that blackfly growth rates were determined by food and temperature. Since temperature was the same in control and enriched sections of the stream, we concluded that *Prosimulium* was responding to increased food supply. At the same time, the midge *Orthocladius (Euorthocladius)* had either pupated or emerged at the downstream site but were still in the last larval instar at the upstream site.

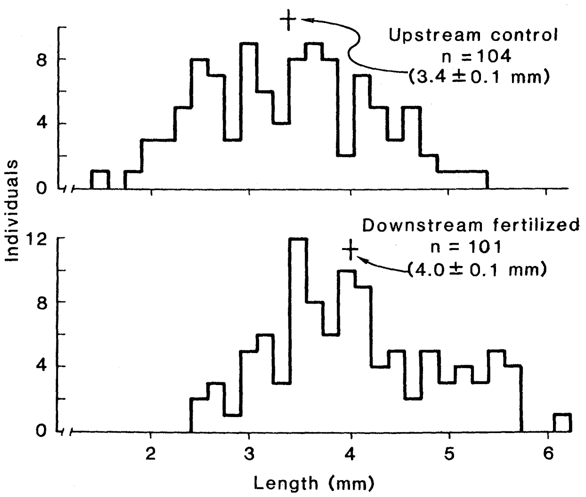

Fig. 3. The size-frequency distribution of a blackfly, *Prosimulium* sp., upstream and 340 m downstream of the phosphorus addition site 23 days after the start of enrichment.

In conclusion, the experiment illustrates that this arctic stream was phosphorus limited and that a few of the algal species responded quickly to enrichment. The undisturbed Kuparuk River appeared to be heterotrophic with dissolved and particulate material from the peaty tundra soils as the predominant sources of organic matter (*24*). However, the growth of the algae when phosphorus was added rapidly changed the Kuparuk from heterotrophy to autotrophy. Growth of the bacteria on the rocky stream bottom was dependent on algal growth and was greatly accelerated by enrichment. Organic matter from the tundra presumably continued to be used at a similar rate in the enriched and control segments, whereas the labile organic matter produced by algae represented a readily utilizable resource for the bacteria. The result was a large increase in bacterial numbers and growth. In contrast, the bacteria growing on particulate organic matter in the stream were little affected by the phosphorus enrichment except when the organic matter was extremely resistant to decomposition. The higher trophic levels, in this case insect larvae, were evidently energy-limited as shown by their response of accelerated growth or development when the algal growth increased in the phosphorus-enriched section. The organic carbon cycle in the river was greatly accelerated by the addition of phosphate alone.

References and Notes

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6. Definitions: epilithon—the microbial community (autotrophic and heterotrophic) attached to stones, sometimes referred to as the slime community or biofilm; epilithic—the adjective derived from epilithon; oligotrophic—a low productivity, nutrient-poor ecosystem [R. G. Wetzel, *Limnology* (Saunders, Philadelphia, 1975)].
7. Chlorophyll on rocks was sampled by brushing the rock surface inside a cylinder sealed against the rock. The material was concentrated on a Whatman GF/C filter and its chlorophyll content measured as described by C. J. Lorenzen [*Limnol. Oceanogr.* **12**, 343 (1967)].
8. This was the result of a single fractionation because of the time taken for this process (12 hours), but there were three analytical replicates. The 0.7-μm filtration was through a combusted Whatman GF/F fiber glass filter while the molecular fractionations were done with Amicon ultrafilters. Thus the 0.7-μm to 300,000 molecular weight fraction could contain colloidal particles (including bacteria) as well as very high molecular weight compounds. But since bacterial densities did not increase (B. J. Peterson *et al.*, unpublished data) through the study section, the increase in organic carbon is most likely due to nonliving organic matter.
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10. We counted only diatoms but undoubtedly other algal groups were affected by the enrichment as well.
11. M. A. Lock and T. E. Ford, *Appl. Environ. Microbiol.* **46**, 463 (1983); since simultaneous measurements of heat output had to be made on the beads colonized under control and enriched conditions, we were unable to replicate as we only had two calorimeters. Replication through time was considered unsuitable as the minimum time for one measurement plus cleanup was 12 hours. However, previous work had shown that duplicate measurements in a United Kingdom river agreed to within 10 percent. In addition, the large differences (>300 percent) between the

activity in the enriched and control light tubes and the agreement between the results from the bioassay tubes and the river support our conclusion that the differences are real.
12. The method is essentially that of K. Gocke [*Mar. Biol.* **42**, 131 (1977)] modified for use with undisturbed epilithon. Colonized glass beads were incubated with filtered water from the appropriate bioassay tube containing 0.5 μg/liter ^{14}C-labeled substrate. Assimilation was measured as incorporation of ^{14}C into bacterial biomass on the beads. Respiration was measured as $^{14}CO_2$ released upon acidification. Turnover times (T_t) were calculated by the following equation: $T_t = t/f$ where t is incubation time (hours) and f is (^{14}C uptake + respiration)/(^{14}C added).
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22. After 23 days of enrichment, blackflies were collected from riffles upstream and 340 m downstream of the enrichment sites by washing randomly selected rocks. Blackflies were subsampled from each preserved collection by randomly selecting approximately 100 larvae from a gridded petri dish and measuring body lengths. Larvae from the enriched riffle were significantly larger than those from the upstream riffle ($t(202) = 5.50$, $P < 0.005$). Baseline data from 1978 showed that blackflies from different riffles in the unenriched stream did not differ significantly in body length.
23. R. W. Merritt, D. H. Ross, G. J. Larson, *Ecology* **63**, 1322 (1982).
24. During 1978, 1979, and 1980, B.J.P. and J.E.H. measured carbon flows in the undisturbed river. Net primary production of the epilithic community was 13 g of carbon per square meter per year. Inputs of eroding peat were 200 to 300 g/m^2/year^1 and inputs of DOC were roughly ten times greater than peat inputs. Thus inputs of organic matter from the tundra vegetation were about 100 times greater than primary production in the river and much of the metabolism of the stream biota depended on the utilization of this organic matter from the tundra. This is supported by the data in Fig. 2A that shows that metabolic heat production of epilithic communities which developed in the dark without added nutrient is roughly 70 percent of the activity in the light, which would include algal metabolism as well.
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Increase of Atmospheric Methane Recorded in Antarctic Ice Core

Abstract. *Air entrapped in bubbles of cold ice has essentially the same composition as that of the atmosphere at the time of bubble formation. Measurements of the methane concentration in air extracted by two different methods from ice samples from Siple Station in western Antarctica allow the reconstruction of the history of the increase of the atmospheric methane during the past 200 years.*

B. Stauffer, G. Fischer
A. Neftel, H. Oeschger
Physics Institute,
University of Bern,
3012 Bern, Switzerland

The atmospheric methane concentration is increasing at a rate of about 1.2 to 1.9 percent per year (*1*). This increase is caused by an increase of the emission rate and possibly by a depletion of the concentration of OH radical in the atmosphere. The reaction of these radicals with methane in the gas phase is the dominant sink and determines the atmospheric lifetime. A higher concentration of CH_4 causes a higher temperature on the earth surface due to an increased greenhouse effect of the atmosphere (*2*). Measurements on air extracted from air bubbles of polar ice cores make it possible to investigate the atmospheric CH_4 concentrations of the past. On the basis of such measurements, Craig and Chou (*3*) and Rasmussen and Khalil (*4, 5*) reported a constant atmospheric CH_4 concentration of about half the present value until about 300 years ago. Younger samples indicate an increase to the present value.

We report measurements made on samples from an ice core from Siple Station in Antarctica. These samples largely fill the gap between previous measurements on ice samples and direct measurements on atmospheric air. Samples from Siple Station are well suited for investigating changes of the atmospheric composition in the recent past, having an excellent time resolution because of the high accumulation rate.

During the formation of ice, air is separated from the atmosphere and enclosed in bubbles. At locations with a low mean annual air temperature (<−20°C), ice is formed by sintering of dry cold firn without interaction with meltwater. At Siple Station (75°55′S; 83°55′W) the mean annual air temperature is −24°C and the mean annual accumulation rate is 500 kg m^{-2}. An ice core was drilled in the Antarctic summer 1983–1984 to a depth of 200 m by the Polar Ice Coring Office (Nebraska) and our institute. It is possible to date the ice to an accuracy of about 2 years down to a depth of 144 m by counting seasonal variations of the electrical conductivity.

The enclosure process of air was investigated by measuring open and isolated porosity on several samples (*6*). The enclosure occurs between 64 and 76 m below surface. Assuming that the air is well mixed in the permeable part of the firn down to the transition from firn to ice, the difference between the mean gas age and the age of the surrounding ice is 95 years, and the time interval needed for gas enclosure is about 22 years. Narrow impermeable layers observed at a depth of 68 m and below are, however, already sealing the air below from the atmosphere. This was also indicated by measurements of the CO_2 concentration on the same ice core (*7*). When this effect is taken into account, the difference between the mean gas age and the age of the surrounding ice is estimated to be about 80 to 85 years instead of 95 years.

Air is extracted from the bubbles of ice samples by a melt extraction and a dry extraction technique. For the vacuum melt extraction, ice samples of about 400 g, containing about 10 percent by volume of air, are melted in an evacuated glass container. The escaping gas is collected continuously during the melting process with a Toepler pump into a small glass bulb (*8*). The advantages of the melt extraction method are the extraction efficiency of almost 100 percent and the simplicity. The disadvantage is the presence of water, where production or consumption of methane by microbiological

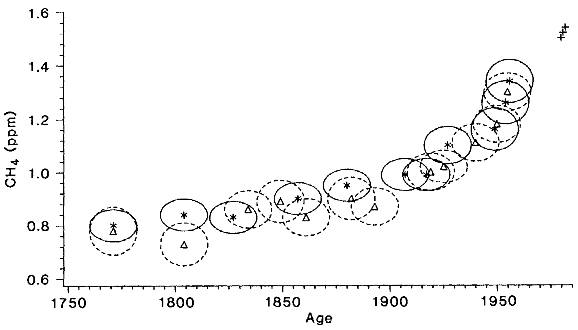

Fig. 1. Measured CH_4 concentration plotted against the estimated mean gas age. Stars with solid ellipses represent results obtained from melt extraction, and triangles with dashed ellipses represent results from dry extraction. The plus signs indicate measurements on atmospheric air (*1*). The vertical semiaxes of the ellipses indicate the estimated precision, and the horizontal semiaxes the duration of the gas enclosure process.