1978-06-01

Nitrogen Transport and Transformations in Canagagigue Creek: Final Report, Project 19A, Agricultural Watershed Studies, Task Group C, Activity 1

International Reference Group on Great Lakes Pollution from Land Use Activities

University of Guelph. Department of Environmental Biology

N. K. Kaushik

J. B. Robinson

L. Chatarpaul

INTERNATIONAL REFERENCE GROUP ON GREAT LAKES POLLUTION FROM LAND USE ACTIVITIES

INTERNATIONAL JOINT COMMISSION

NITROGEN TRANSPORT AND TRANSFORMATIONS IN CANAGAGIGUE CREEK

NITROGEN TRANSPORT AND TRANSFORMATIONS IN CANAGAGIGUE CREEK

Final Report Project 19A

Agricultural Watershed Studies

Task Group C, Activity 1

International Reference Group on

Great Lakes Pollution from Land Use Activities

June 1, 1978

J.B. Robinson, N.K. Kaushik, L. Chatarpaul

Department of Environmental Biology

University of Guelph

3.0 Disclaimer

The study discussed in this document was carried out as part of the efforts of the Pollution from Land Use Activities Reference Group, an organization of the International Joint Commission, established under the Canada - U.S. Great Lakes Water Quality Agreement of 1972. Funding for this study was provided by the Ontario Ministry of Agriculture and Food. Findings and conclusions are those of the investigators and do not necessarily reflect the views of the Reference Group or its recommendations to the Commission.

4.0 Acknowledgements

We are happy to acknowledge the contributions of R. Mason and P. Perk who assumed much of the responsibility for field installations and sampling. The willing and enthusiastic assistance of C.K. Lee and L. Kristoff is also gratefully acknowledged. Helpful advice was provided by many of our colleagues at the University of Guelph, especially by Professor H.R. Whiteley of the School of Engineering, and Professor M.H. Miller, Department of Land Resource Science.

5.0 Table of Contents

6.0 List of Tables

7.0 List of Figures

8.0 Summary

Agricultural activities are known to be a source of appreciable amounts of nitrogen contamination of both surface and ground waters. Much of this nitrogen enters water as nitrate. Fixed nitrogen is very reactive and undergoes a large variety of biological transformations, the kinds and rates of which depend largely on environmental factors such as presence or absence of oxygen, temperature etc. While these processes have been extensively studied in soils and, to some extent in lakes, little is known about their occurrence in streams. The degree to which nitrogen forms are subject to transformation processes while in transport in streams, particularly to sink processes such as denitrification, may significantly affect the degree to which agricultural activities contaminate receiving waters. The objective of this project was to determine the extent of the sink process, denitrification, in streams and to asses its effect on stream transport of N.

Both laboratory and field studies were carried out. In the laboratory, plexiglass columns were used to determine the rate at which nitrate-N disappeared from water overlying stream sediment collected from a number of PLUARG watersheds. It was found that disappearance was almost complete in about 2 weeks for most sediments at 22 C when the starting concentration in the water was 10 mg l^{-1}. Nitrate disappeared less rapidly at lower temperatures but the rate was enhanced considerably when tubificid oligochaete worms were present in the sediment and when metabolizable carbon (in the form of dried leaves) was added to the sediment. When ^{15}N nitrate was used in column experiments it could not be detected after incubation, in any fixed nitrogen fraction in either water or sediment, nor was it evolved as ammonia, and it was concluded therefore that disappearance of nitrate resulted from denitrification in these experiments. The use of ^{15}N also permitted estimation of nitrification rate in the sediment and it was shown

that nitrate was produced in the sediment near the water interface and was then denitrified along with added nitrate, when it diffused below the aerobic zone of the sediment. Extrapolating from these column studies to the stream it appears that for the Canagagigue Creek (Ag 4), Holiday Creek (Ag 5) and the Little Ausable River (Ag 3) the denitrification rate is in the order of 0.14 g m^{-2} of stream sediment surface day^{-1} at 22°C. This is in the same order of magnitude as our previous observations on sediments from another S. Ontario stream and also with estimates made recently for certain European stream sediments.

Field studies of nitrogen transport in a reach of the East branch of the Canagagigue Creek (near Ag 4) were complicated by the presence of many groundwater seeps, tile outlets and small tributaries in that watershed. High concentrations of nitrate-N are a characteristic of surface flows in the watershed however, and tiles were found to flow year-round with concentrations of nitrate-N in the range of 20 mg l^{-1}. The establishment of a nitrogen budget was found to be impossible for short reaches for long periods of time because of problems in adequately quantifying flow. However, the crude figures obtained for each of 18 days in 1976 showed losses ranging from 1.3% to 18.8% of the input nitrate-N for the reach studied. On one of these days there appeared to be a small gain of nitrate-N over the reach. The median loss observed for the 18 days was about 7.5% of the input nitrate-N while the mean was 8.1%.

It can be assumed that the removal of nitrate-N under stream conditions will be greater than the removal under laboratory conditions because of turbulence and sediment mixing by benthic activities. It is proposed therefore that a factor of 0.2 g m^{-2} d^{-1} could be applied to calculate field losses of N during transport in streams during summer. A correction for cooler temperatures is recommended.

9.0 Introduction

In recent years it has been observed that some of the nitrate-N entering streams is lost from water, presumably as a result of denitrification in the sediment (Kaushik and Robinson, 1976). This loss depends, among other factors, upon quality and quantity of organic matter and on water temperature. Thus all the nitrate-N received in streams from non-point sources as a result of agricultural or other land use activities may not actually reach lakes. It was with this background that the present project on Canagagigue Creek, draining PLUARG watershed AG-4, was started. The primary aim of the project was to determine whether nitrate-N in transport in the stream was denitrified, and if so, at what rate. The project was divided into two components, one dealing with laboratory studies and the other comprising field investigations. The prupose of laboratory studies was to determine whether sediments from the two branches of the stream promoted nitrate loss from overlying water and through the use of labelled nitrate-N, to elucidate the exact mechanism of such loss. In field investigations, it was intended that a selected reach of Canagagigue Creek be studied in order to calculate short term nitrogen budgets and determine rates of nitrate-N loss from the stream.

The study was conducted in the upper basin of Canagagigue Creek, near Floradale, Ontario (Fig. 1), where two main branches can be identified. The West branch is the PLUARG watershed AG-4 and is characterised by its marked seasonal flow, its eroded banks and by the virtual absence of deciduous vegetation throughout its length. We observed that the West branch was dry for most of the summer and was therefore unsuitable for our purpose. The East branch, on the other hand, is perennial, has a more defined channel and flows through areas of developed farmland interspersed with mixed deciduous and coniferous bush. Field and laboratory

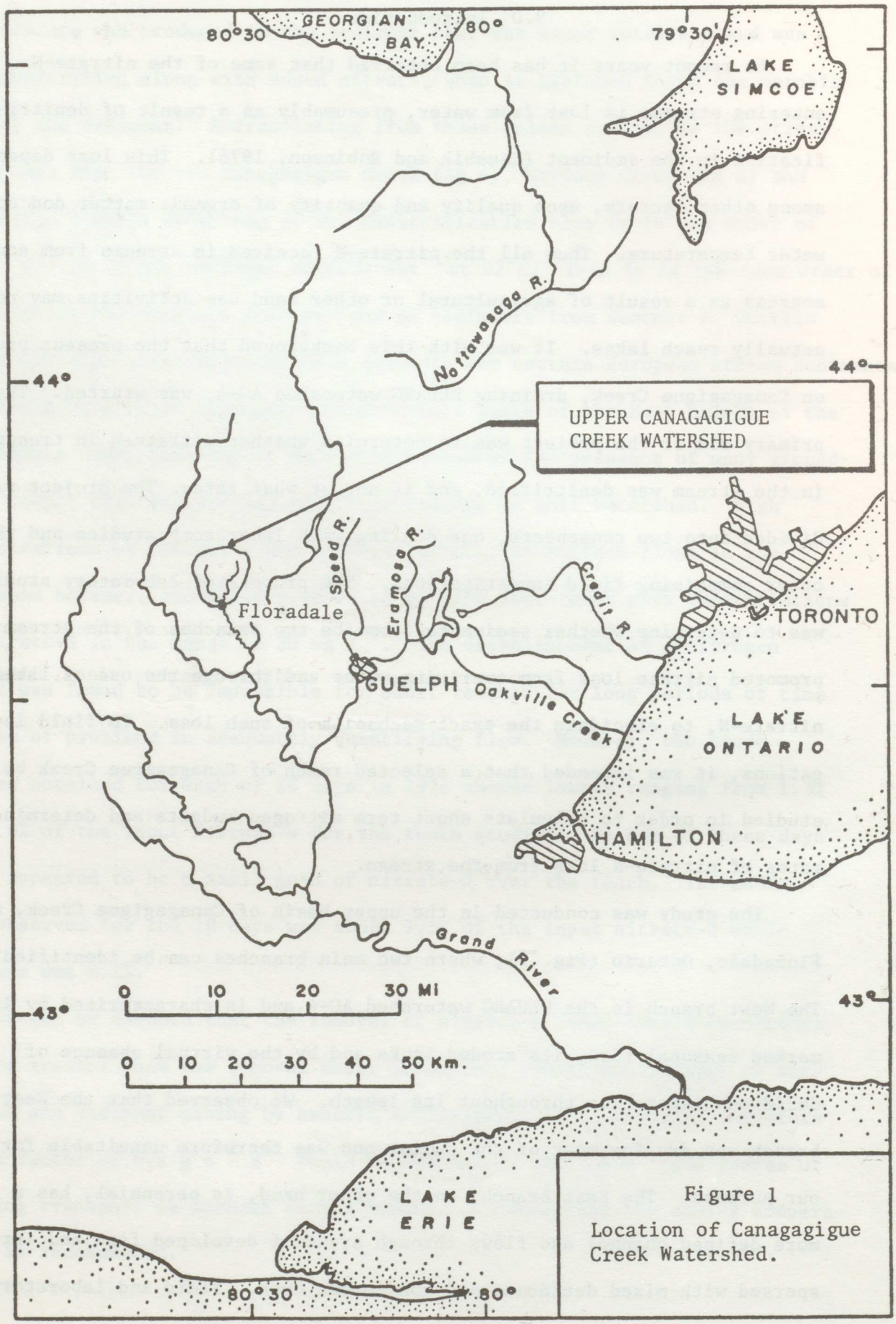

Figure 1
Location of Canagagigue Creek Watershed.

studies were, therefore, concentrated on the East branch, although it was rather more complex than we had hoped for. Comparative laboratory studies were also carried out on sediments from Holiday Creek (Ag. 5), a tributary of the Middle Thames River, situated in Oxford County and Little Ausable River (Ag. 3), a part of the Ausable River drainage basin, located in Huron and Perth Counties.

10.0 Methods

10.1 Laboratory Studies:

Stream sediment was collected by using a 60 cm long plexiglass column of 5.60 cm ID and 0.40 cm wall thickness. One end of the column was sharpened to facilitate penetration. In the field, the sharpened end of the column was pushed about 10 cm into the sediment, its top end was closed with a rubber stopper and the column was pulled out with the intact core of sediment. The lower end was then plugged with a stopper. For some experiments such intact cores were used, while in others, sediment collected in this manner was mixed and columns containing a 10 cm depth of the mixed sediment were prepared as outlined below. All sediment samples were stored in a refrigerator at $2^{o}C$ and used within two days of collection.

For the columns packed with mixed sediment, sediment samples were thoroughly mixed in a plastic bucket and added to plexiglass tubes closed at the lower end with rubber stoppers. This procedure eliminated likely variation in the physico-chemical properties of the sediment of different columns. Air bubbles were purged by a light tapping of the columns and adhering sediment was washed from the sides of the column walls with deionised water.

After the sediment columns had settled overnight at $2^{o}C$, the supernatant water was siphoned off. To each column 500 ml of solution containing about 10 mg l^{-1} nitrate-N as KNO_3 was added very slowly to avoid disturbing the sediment. Controls consisted of replicate sediment columns overlain with deionised water and columns containing only 500 ml of the KNO_3 solution (no sediment).

The liquid layer in completed columns was about 20 cm deep. A gas mixture of 21% oxygen and 79% helium was introduced by a sparger situated about 1 cm above the sediment-water interface. This procedure was intended to create a little turbulence and to keep the supernatant solution aerated in an effort to simulate stream conditions. For low temperature incubations, the columns were placed in dark temperature-controlled environmental chambers. Columns covered with dark plastic were kept in the laboratory for room temperature experiments. Details of columns are shown in Fig. 2.

For experiments requiring the presence of worms, the sediment was first visually inspected for their natural occurrence. Any worm found was removed by suction applied to a pasteur pipet. The light colored sandy nature of the Canagagigue sediment usually facilitated this process. All the columns were packed with this "worm-free" sediment and from these, no-worm controls were selected. To each of additional replicated sets of columns, oligochaete worms from a culture were added to give a density equivalent to 15,000 worm m^{-2} of sediment surface.

To provide a large and continuous supply, the worms used in the experiments were obtained from Toronto Harbour, near Hanlan's Point. The worms were taken in the sediment under about 5 m of water with an Ekman grab and sieved in the field in a wash bucket of 0.5 mm pore size. The residues were placed in large plastic pails with lake water and stored in a controlled environmental chamber at $10^{o}C$. Before the worms were used in the column experiments, they were picked with a pasteur pipet and rinsed twice in deionised water to remove any sediment or adhering particles.

Nitrate-N concentration in the supernatant was monitored routinely by removing 2.5 ml samples and analysing for NO_3-N--using the modified chromotrophic acid method (West and Ramachandran, 1966 and APHA, 1971). Spot

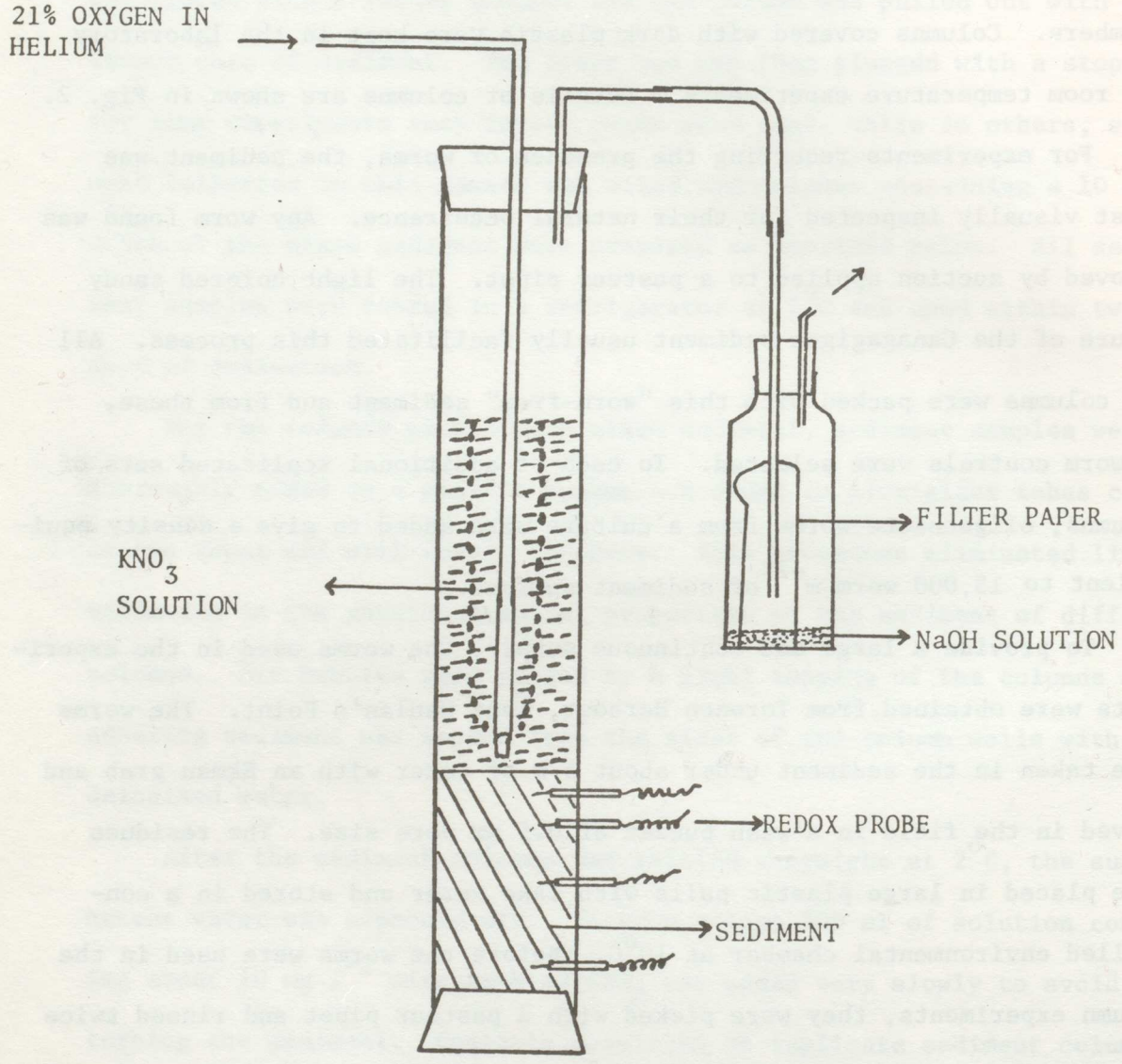

Figure 2: Details of columns for laboratory experiments.

tests for the presence or absence of nitrite-N were made according to the method of Rider and Mellon (1946) modified for qualitative tests.

Ammonium-N and organic-N in the overlying solution were determined by a macro-Kjeldahl procedure (Bremner, 1965 a and b and APHA, 1971). The 500 ml supernatant solution was placed in a 800 ml flask and the free ammonium-N was determined by distillation with MgO. About 200 ml of the distillate was collected in a 500 ml flask containing 50 ml methyl red-methylene blue indicating boric acid (2%). The remaining solution was used to estimate the organic-N content by a regular macro-Kjeldahl digestion with concentrated sulfuric acid, potassium sulfate and mercuric oxide followed by distillation with sodium hydroxide-thiosulfate solution. All titrations were done using 0.01 N sulfuric acid and N was calculated on the basis of 1 ml 0.01 N H_2SO_4 = 0.14 mg ammonium-N.

Ammonium-N and nitrate-N in the sediment were determined on a potassium chloride extract. The extract was prepared by shaking the wet sediment in 2M KCl (10:1 W/V) for one hour followed by filtration on glass fiber filter paper (Reeve-Angel). Ammonium-N and soluble organic-N were estimated by the macro-Kjeldahl procedure described above. Nitrate-N (including nitrite-N) was then determined by distillation after conversion to ammonium-N with finely ground Devarda Alloy. Organic-N content in the sediment was measured by the macro-Kjeldahl method (APHA, 1971). A sediment suspension was prepared by adding 400 ml of deionised water to a 800 ml digestion flask containing 0.5 g of finely ground air-dried sediment sample. The digestion, distillation and titration procedures described above were then followed.

The combustible matter content of the sediment was estimated by using the weight loss by combustion at 500°C for 4 hours. Carbon content was assumed to be 50% of the loss on ignition. The available carbon in the sediment was measured by a wet conbustion procedure using anthrone and adapted from Burford

and Bremner (1975).

The evolution of CO_2 from the columns was quantified and used to estimate the carbon loss during incubation according to a method described by Sain and Broadbent (1977). Vials containing 2.5 ml of 1N NaOH with a filter paper "wick" were inserted on the gas outflow line of the columns. Periodically the contents of the vials were analysed for CO_2 by adding 3.5 ml of $BaCl_2$ solution (1N) to precipitate the carbonates. The excess NaOH was titrated with HCl solution using phenolphthalein as indicator. Carbon loss was calculated by the formula:

$$C = 6 \times N \times (V_1 - V_2)$$

where C = mg of Carbon loss

N = normality of HCl used

V_1 = volume of HCl used for blank

V_2 = volume of HCl used for sample

The chloride concentration in solution was determined by the standard method (APHA, 1971) using silver nitrate and potassium dichromate.

For experiments using labelled nitrogen, analyses for ammonium-, nitrate- and organic-N were performed according to methods described above. The first 50 ml of each distillate was saved for ^{15}N determination. These samples were first redistilled with NaOH on a special nitrogen -15 apparatus modified for easier cleaning (supplied by Eck and Krebs Co., N.Y.) and 5-10 ml of distillate was collected in excess 0.1 N H_2SO_4. This procedure concentrated the samples and eliminated the indicating boric acid solution.

The ^{15}N isotope ratio analyses were performed on an optical emission spectrometer (Jasco N1A-1 ^{15}N Analyser) using the general method described by Fiedler and Proksch (1972 and 1975), modified by Liao (1976) and specific procedures recommended by Japan Spectroscopic Co. Ltd.

The Dumas dry conbustion method was employed to convert the nitrogen samples to dinitrogen gas. Pyrex discharge tubes 200 mm long x 6 mm OD with a centre

constriction measuring 25 mm long and 3 mm OD were supplied by Scientific Glassware, Hamilton, Ontario and the University of Guelph Glass Blowing Section, and used for evacuation and combustion procedures. Quartz wool plugs were placed at both ends of the constriction. Approximately 10 µg of the acidified N sample was placed on previously combusted 2.5 cm glass fiber filter paper (Whatman GF/C) with the aid of a micropipet and dried in a 40°C oven. One end of the discharge tube was sealed after receiving the sample and 0.5 g of a mixture of CuO and cuprox-platinum catalyst (Coleman Instruments, Illinois). A 1 gm briquette consisting of alumina and calcium oxide (1:1) combusted at 800°C for 2 hrs was placed in the other end. This briquette was compressed on a pellet press (Parr Instrument Co.) at 15 p.s.i. The unsealed end of the discharge tube was attached to a high vacuum line by means of a Swagelok union and Teflon ferrules (Niagara Valve Co.). The tube was then evacuated until a vacuum of 10^{-4} torr was indicated. At this point, the other end of the tube was sealed with a hand torch. The sealed tubes were combusted at 550°C in a muffle furnace for 8 hrs before analyses for ^{15}N abundance were carried out.

The sample preparation procedures outlined above were standardized by using samples of known ^{15}N abundance. These standard samples were analysed using a mass spectrometer (Atlas GD-150) by Prof. E.A. Paul, Soils Dept., University of Saskatchewan. One standard was included in every batch of samples to guarantee the efficacy of the procedures. The ^{15}N analyser was calibrated each time using a Jasco-prepared series of discharge tubes containing nitrogen gas of known ^{15}N abundances. In this way a calibration curve was established and the absolute ^{15}N values of the unknown samples determined. The ^{15}N abundance in the sample was calculated from the peak heights on the recorder chart corresponding to ^{28}N and ^{29}N molecules using the formula (Fiedler and Proksch, 1975):

$$A = \frac{100}{2 \cdot R + 1}$$

where A = atom-percent abundance ^{15}N

and R = ratio of the ^{28}N and ^{29}N peak heights.

The ^{15}N excess was obtained by subtracting the background ^{15}N abundance (0.36 A%) from the sample ^{15}N abundance.

The probes used to measure redox potentials in the sediment were prepared as described by Whisler et al. (1974) and blacked by the method of Quispel (1947). Each probe was then passed through a hole in a #0 rubber stopper so that the tip of the probe was positioned at about the centre of the column (Fig. 2). The rubber stopper was fitted tightly in a hole drilled in the side of the column at an appropriate depth in the sediment column. A lead from each redox probe was soldered to a terminal on a multi-point switch to which was connected a pH meter with a standard reference calomel electrode. The redox reading was obtained by placing the reference electrode in the overlying water and the switch was moved until all the probes were read.

A Tektronic 4051 Computer was employed to perform the statistical analyses of the data. Linear and polynomial regression and correlation analyses and the 2-way analysis of variance (ANOVA) procedure were the principal programs used (Steel and Torrie, 1960).

10.2 Field Studies:

Grab samples were taken weekly in 1 liter polypropylene bottles from a large number of sites in the East branch and from two sites on the West branch (Fig. 3). Water temperature at each site was also recorded. The samples were brought back to the laboratory and analysed for ammonium-, nitrate- and organic-N as described in the preceding section. A reach of the East branch, between sampling sites 3 and 7, was selected for budgeting purposes. Flowrate at station 3 was measured by means of a staff gauge in 1976, and by an Ott meter in 1977. Flow at station 7 was taken from the record maintained by the Water

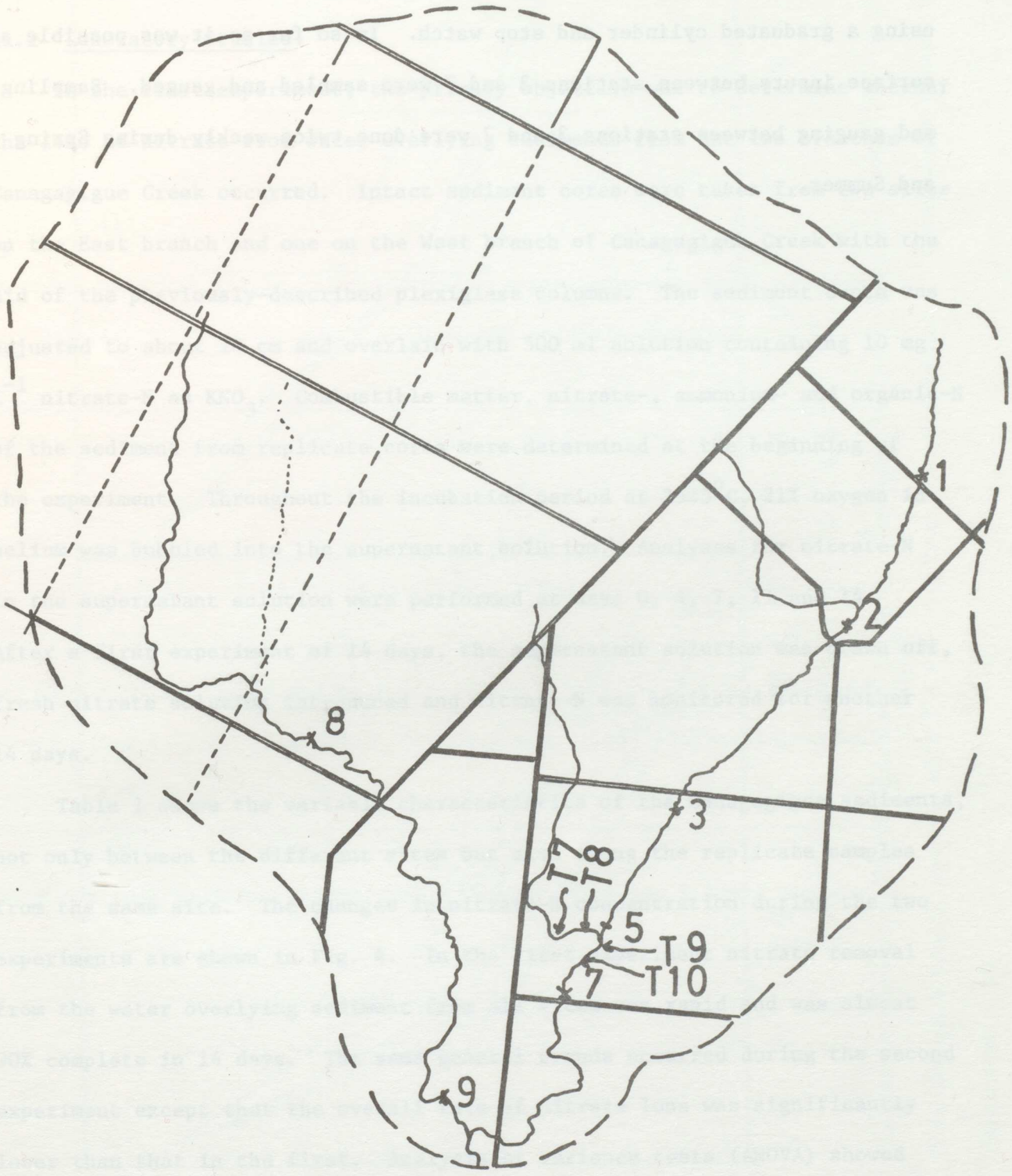

Figure 3: Location of sampling stations and tiles on the main branches of Canagagigue Creek.

Survey of Canada at that site. The flow from tile outlets was measured by using a graduated cylinder and stop watch. In so far as it was possible all surface inputs between stations 3 and 7 were sampled and gauged. Sampling and gauging between stations 3 and 7 were done twice weekly during Spring and Summer.

11.0 Results

11.1 Laboratory Studies:

In the first experiment, the primary objective was to determine whether the loss of nitrate from water overlying sediments from the two branches of Canagagigue Creek occurred. Intact sediment cores were taken from two sites on the East branch and one on the West branch of Canagagigue Creek with the aid of the previously-described plexiglass columns. The sediment depth was adjusted to about 10 cm and overlain with 500 ml solution containing 10 mg l^{-1} nitrate-N as KNO_3. Combustible matter, nitrate-, ammonium- and organic-N of the sediment from replicate cores were determined at the beginning of the experiment. Throughout the incubation period at 25±3°C, 21% oxygen in helium was bubbled into the supernatant solution. Analyses for nitrate-N in the supernatant solution were performed at days 0, 4, 7, 11 and 14. After a first experiment of 14 days, the supernatant solution was drawn off, fresh nitrate solution introduced and nitrate-N was monitored for another 14 days.

Table 1 shows the variable characteristics of the Canagagigue sediments, not only between the different sites but also among the replicate samples from the same site. The changes in nitrate-N concentration during the two experiments are shown in Fig. 4. In the first experiment nitrate removal from the water overlying sediment from all sites was rapid and was almost 90% complete in 14 days. The same general trends occurred during the second experiment except that the overall rate of nitrate loss was significantly lower than that in the first. Analysis of variance tests (ANOVA) showed there was no difference ($P < 0.01$) in the rate of nitrate loss due to sediment sampling location.

Surprisingly, the average nitrate loss from columns containing West branch sediment was in the same order of magnitude as that of the more highly

TABLE 1: Some characteristics (air-dry basis) of the sediment taken from different sites on Canagagigue Creek.

	SITES		
	1[1]	2	3
pH	7.3	7.2	7.2
NH_4-N (ppm)	28.0 ± 7.0[2]	21.0 ± 7.0	14.0 ± 3.0
NO_3-N (ppm)	0	0	0
Org-N (%)	0.31 ± 0.04	0.30 ± 0.04	0.20 ± 0.03
Combustible Matter (%)	10.5 ± 2.9	8.4 ± 2.5	4.6 ± 1.1
C:N	17:1	14:1	12:1

[1] Sites 1 and 2 are located on the East branch and Site 3 on the West branch

[2] Mean of 3 replicates ±SD.

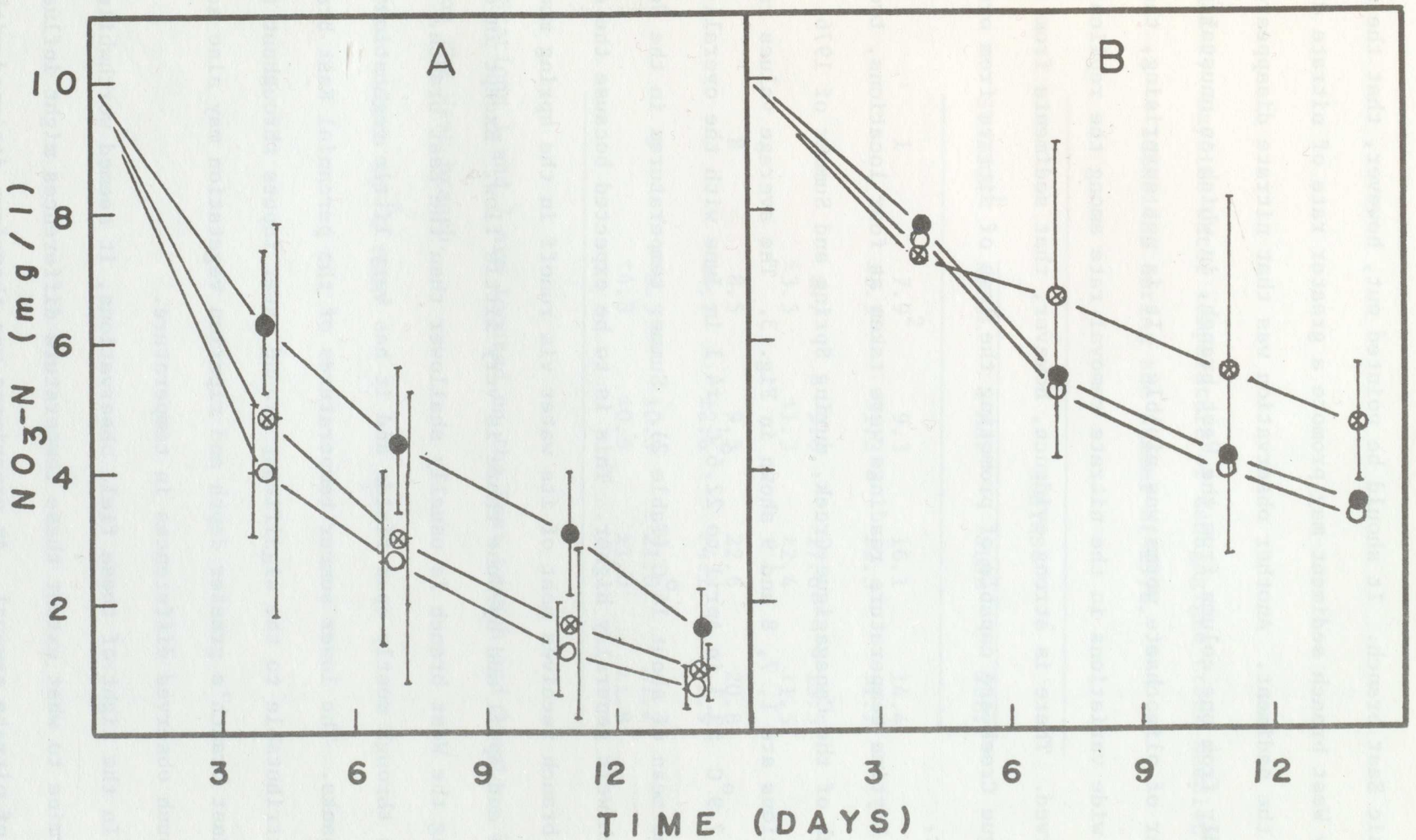

Figure 4: A. Changes in concentration of nitrate-N in water overlying sediment from two sites (⊗ —— ⊗ , ● —— ●) from the East branch and one site (o —— o) from the West branch of Canagagigue Creek. Mean of 3 replicates ± SD.

B. Represents concentration changes when fresh nitrate solution was added to columns.

organic East branch. It should be pointed out, however, that the more sandy West branch sediment may promote a greater rate of nitrate diffusion into the sediment. Another observation was that nitrate disappeared more rapidly from one column from the West branch, in which an unusually large number of oligochaete worms was visible. It is not surprising, then, that such wide variations in the nitrate removal rate among the replicates were observed. There is strong evidence, however, that sediments from the Canagagigue Creek are capable of promoting the loss of nitrate from overlying water.

Daytime temperature readings were taken at four locations, two on each branch of the Canagagigue Creek, during Spring and Summer of 1976. These locations are 1, 7, 8 and 9 shown in Fig. 3. The average values ranged from 7.9°C ±3.3 in April to 22.6°C ±4.1 in June with the overall four months mean of about 14°C (Table 2). Summer temperatures in the West branch were generally higher. This is to be expected because the ephemeral West branch receives most of its water via runoff in the spring months of March and April and by June there is very little flow. Except in early spring the West branch is usually shallower than the East branch. It flows through mostly open fields and it has very little vegetation along its banks. The lower summer temperatures of the perennial East branch may be attributable to the significant ground water inputs throughout the year. The East branch's greater depth and riparian vegetation may also account for such observed differences in temperature.

In the light of these field observations, it seemed worthwhile to determine to what extent these temperature differences might influence the rate of nitrate removal. An experiment was therefore designed with such an objective in view.

Sediment was taken from the East branch (near station 1, Fig. 3) and

TABLE 2: Daytime water temperature of Canagagigue Creek sampled at 4 locations during the Summer of 1976.

STATION[1]	MONTHS			
	APRIL	MAY	JUNE	JULY
1	7.9[2] ±3.3	9.3 ±1.3	16.1 ±2.3	14.4 ±1.1
7	8.7 ±3.5	9.8 ±1.3	17.6 ±2.4	15.5 ±1.5
8	8.5 ±4.7	9.5 ±2.6	22.6 ±4.1	20.8 ±2.9
9	8.3 ±4.8	10.5 ±0.5	21.0 ±1.0	21.2 ±1.8

[1] Stations 1 and 7 are located on the East branch and Stations 8 and 9 on the West branch.

[2] Mean values in ^{o}C ±SD.

was thoroughly mixed to minimize the variation in the physico-chemical characteristics of sediment of replicate columns. The sediment possessed similar characteristics to that of site 1 of Table 1. Triplicate columns with a 10-cm sediment layer overlain with 500 ml of solution containing 12 mg l^{-1} nitrate-N as KNO_3 were incubated in controlled environmental chambers at 7°C, 15°C and 22°C. After 9 days of routine analysis for nitrate in the supernatant, fresh nitrate solution was introduced in the columns that were previously incubated at 15°C and 22°C and these columns were monitored for a second experimental period at the same temperatures. The gas mixture of 21% oxygen and 79% helium was bubbled into the supernatant for the duration of both experiments.

The changes in nitrate-N concentration during the two experiments are shown in Fig. 5. In the first experiment, nearly 50% of the added nitrate-N was lost in 9 days at 7°C. During the same period the nitrate-N lost at 15°C and 22°C was about 80% and 90% respectively. ANOVA tests show that the differences in nitrate-N loss at 7°C, 15°C and 22°C were significant ($P < 0.01$). In the second experiment, about 60% of the nitrate was lost in 9 days at 15°C and this loss was not significantly different ($P < 0.01$) from the 22°C incubation.

The results of this experiment show that temperature influences the removal rate of nitrate in aerated water overlying stream sediment: the process proceeding more rapidly at higher temperatures. The slowing of this rate upon repeated additions of nitrate to the same sediment in this and the previous experiment is evidence that metabolizable carbon content may also be a controlling factor or, as will be shown later, that nitrate is being produced.

During the course of preliminary experiments, it was observed that certain individual columns always lost nitrate much more rapidly than did other

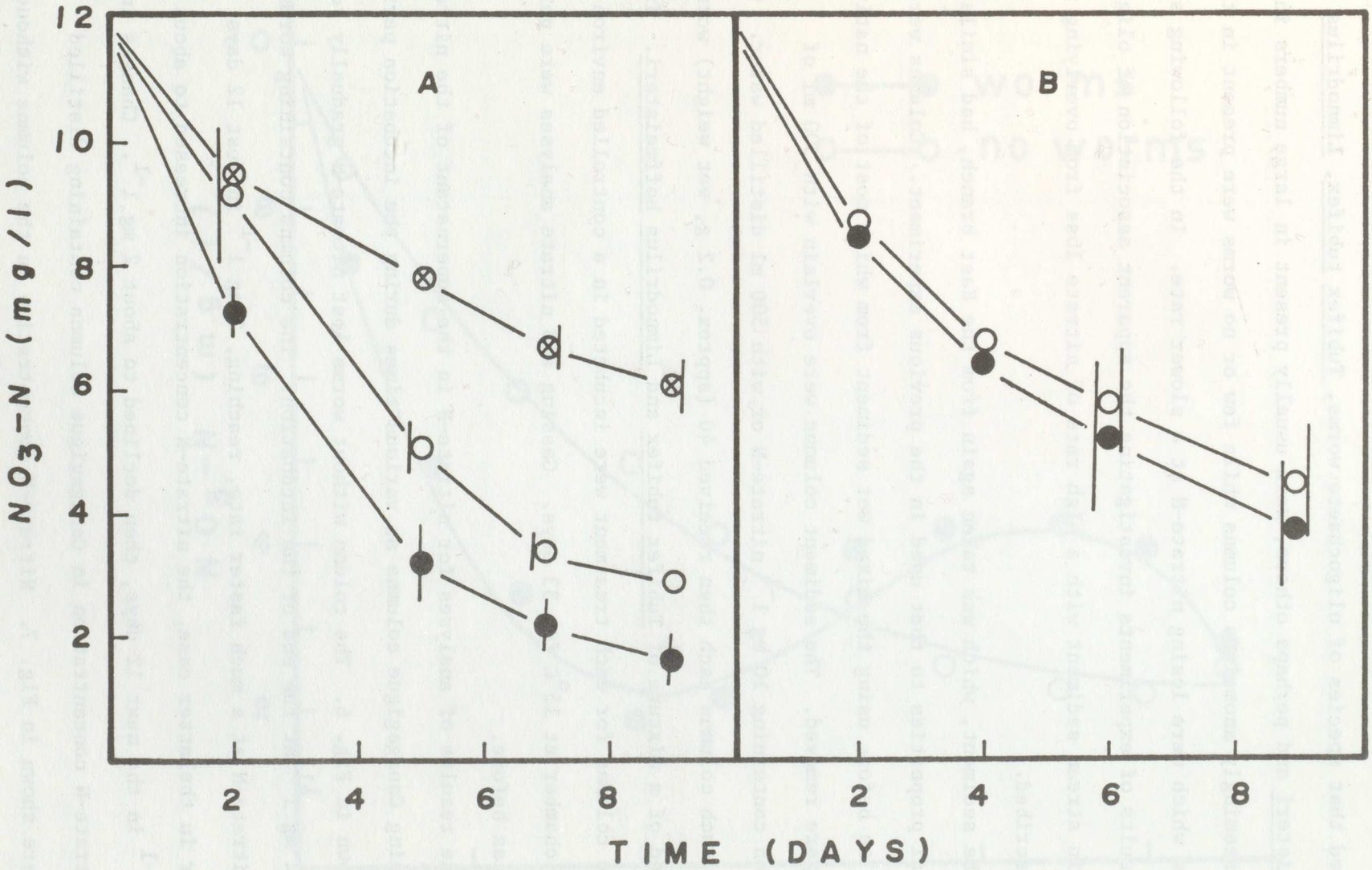

Figure 5: A. Changes in concentration of nitrate-N in water overlying Canagagigue sediment in columns maintained at 7°C (● —— ●), 15°C (O —— O) and 22°C (● —— ●). Each point is the mean of 3 replicates ±SD.

B. Represents concentration changes when fresh nitrate was added to columns at 15°C and 22°C.

replicates of the same treatment. Closer examination of the columns revealed that species of oligochaete worms, Tubifex tubifex, Limnodrilus hoffmeisteri and perhaps others, were usually present in large numbers in these seemingly anomalous columns while few or no worms were present in the columns which were losing nitrate-N at a slower rate. In the following section, the results of experiments investigating the apparent association of oligochaete worms in stream sediment with a high rate of nitrate loss from overlying water, are described.

The sediment, which was taken again from the East branch, had similar chemical properties to that used in the previous experiment. Columns were packed as before using the mixed wet sediment from which most of the native worms were removed. The sediment columns were overlain with 500 ml of solution containing 10 mg l^{-1} nitrate-N or with 500 ml distilled water. One set of such columns each then received 40 (approx. 0.2 g, wet weight) worms composed of a mixture of Tubifex tubifex and Limnodrilus hoffmeisteri. Triplicate columns for each treatment were incubated in a controlled environmental chamber at 15°C for 33 days. Gassing and nitrate analyses were performed as before.

The results of analyses for nitrate-N in the supernatant of the nitrate-containing Canagagigue columns at various times during the incubation period are shown in Fig. 6. The column without worms lost nitrate-N gradually to about 2 mg l^{-1} at the end of the incubation. The columns containing worms lost nitrate-N at a much faster rate, reaching, 2 mg l^{-1} in just 12 days. However in thelatter case, the nitrate-N concentration increased to about 4 mg l^{-1} in the next 12 days, then declined to about 2 mg l^{-1}. Changes in the nitrate-N concentration in Canagagigue columns containing distilled water are shown in Fig. 7. Nitrate-N concentration in the columns without worms increased to a maximum of about 3 mg l^{-1} in 20 days. In the columns

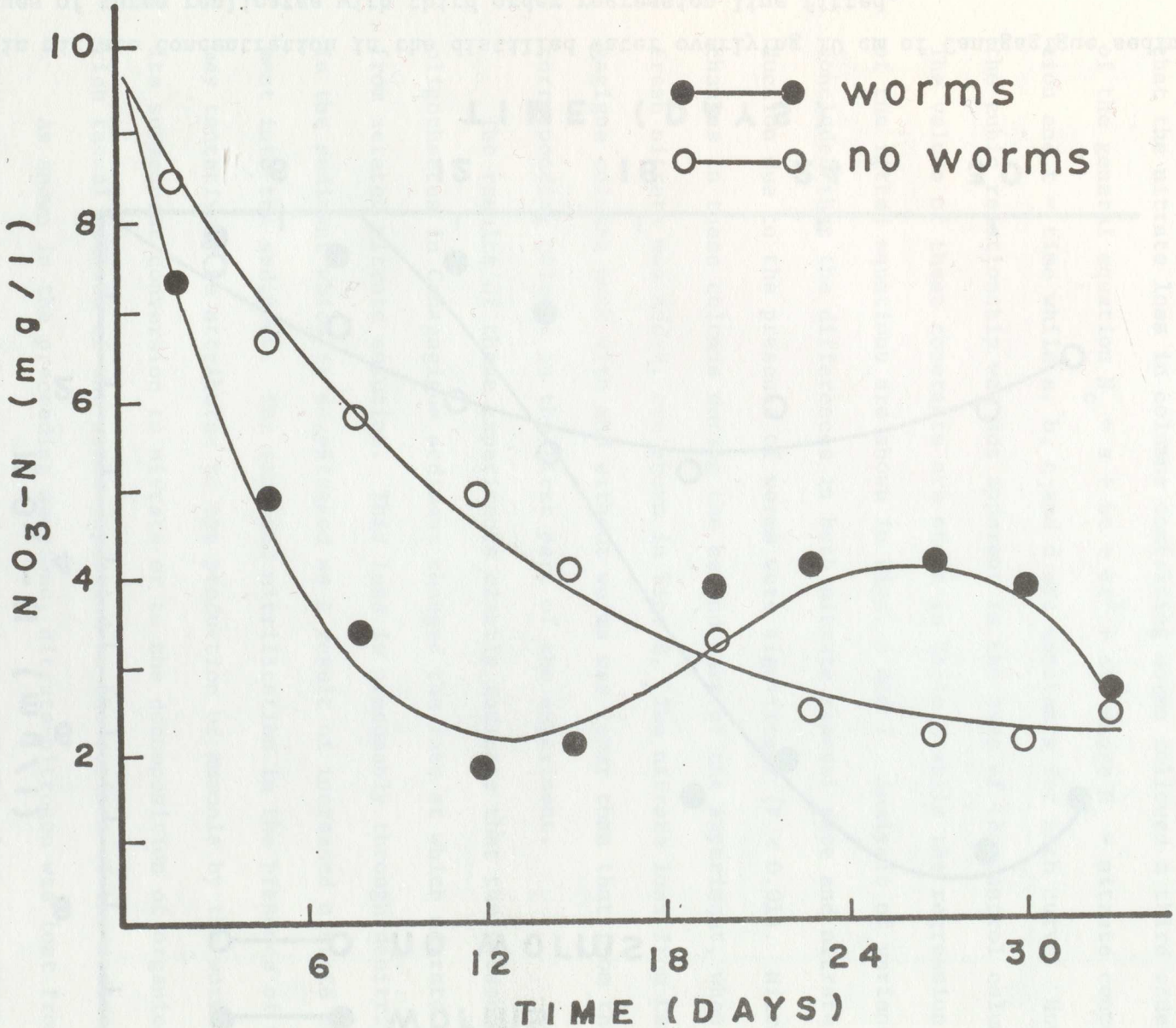

Figure 6: Changes in nitrate-N concentration in the nitrate solution overlying 10 cm of Canagagigue sediment. Mean values of three replicates with third order regression line fitted.

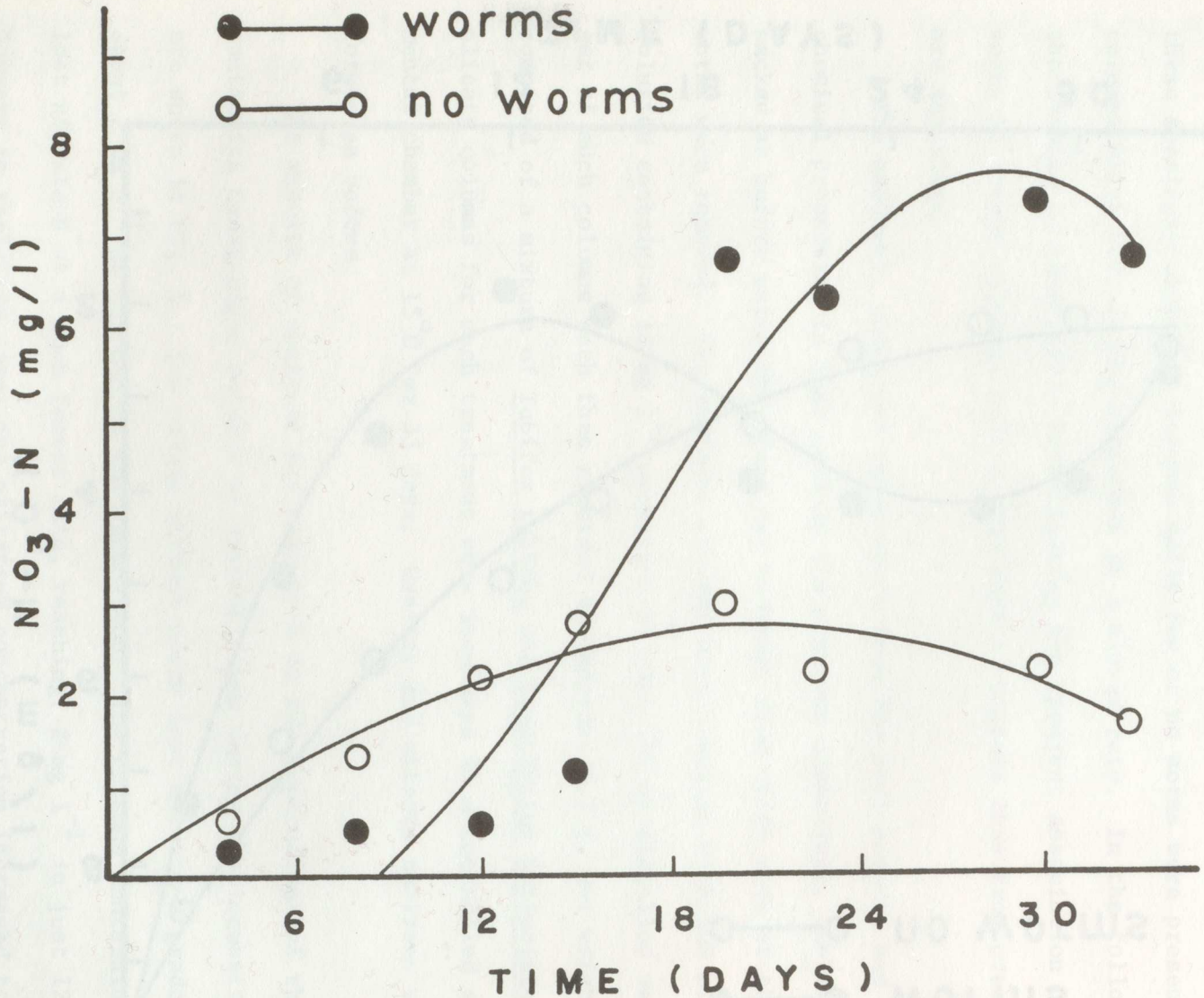

Figure 7: Changes in nitrate concentration in the distilled water overlying 10 cm of Canagagigue sediment. Mean values of three replicates with third order regression line fitted.

containing worms, nitrate-N increased sharply after 12 days to a peak of 7 mg l^{-1} in 20 days. Regression and correlation analysis of the data showed that the nitrate loss in columns containing worms followed a third order function of the general equation $N_c = a + bt + ct^2 + dt^3$ where N_c = nitrate concentration and t = time while a, b, c and d are constants for each curve. However, the cubic relationship was not apparent in the case of the control columns. The values of these constants are shown in Table 3 while the regression lines of the fitted equations are shown in Figs. 6 and 7. Analysis of variance tests concluded that the differences in both nitrate removal rate and nitrate production due to the presence of worms were significant ($P < 0.01$). Nitrate changes in these columns during the second part of the experiment, when fresh nitrate was added, are shown in Fig. 8. The nitrate loss from Canagagigue columns both with and without worms was lower than that from the corresponding columns in the first part of the experiment.

The results of these experiments clearly indicate that the presence of oligochaetes in Canagagigue sediment changed the rate at which nitrate is lost from aerated nitrate solution. This loss is presumably through denitrification in the sediment which is accentuated as a result of increased nitrate movement into the sediment. The enhanced nitrification in the presence of worms may tentatively be attributed to the production of ammonia by the worms and its subsequent conversion to nitrate or to the decomposition of organically rich faecal pellets of the worms deposited on the surface of the sediment.

As shown in the preceeding sections, nitrate-nitrogen was lost from aerated solution overlying Canagagigue sediment and this loss was assumed to have proceeded via microbial denitrification. The following series of experiments was designed to established this pathway of loss unequivocally, and to accomplish this, the use of isotopically labelled nitrate-nitrogen became necessary.

TABLE 3: The values of the coefficients a, b, c and d of equations with the general form $N_c = a + bt + ct^2 + dt^3$ fitted to data (Figs. 6 and 7) for NO_3-N changes in water above stream sediment.

SUPERNATANT SOLUTION	TREATMENT	COEFFICIENTS a	b	c	d	r^2
NO_3-N	no worms	9.697[1] ±0.200	-0.587 ±0.059	0.015 ±0.004	-0.00010 ±0.00009	0.98
	worms	10.100 ±0.180	-1.503 ±0.054	0.088 ±0.004	-0.00150 ±0.00008	0.98
H_2O	no worms	-0.182 ±0.180	0.261 ±0.059	-0.005 ±0.004	-0.00003 ±0.00009	0.86
	worms	0.558 ±0.561	-0.508 ±0.160	0.061 ±0.012	-0.00122 ±0.00023	0.91

[1] Mean values of three replicates ±SE.

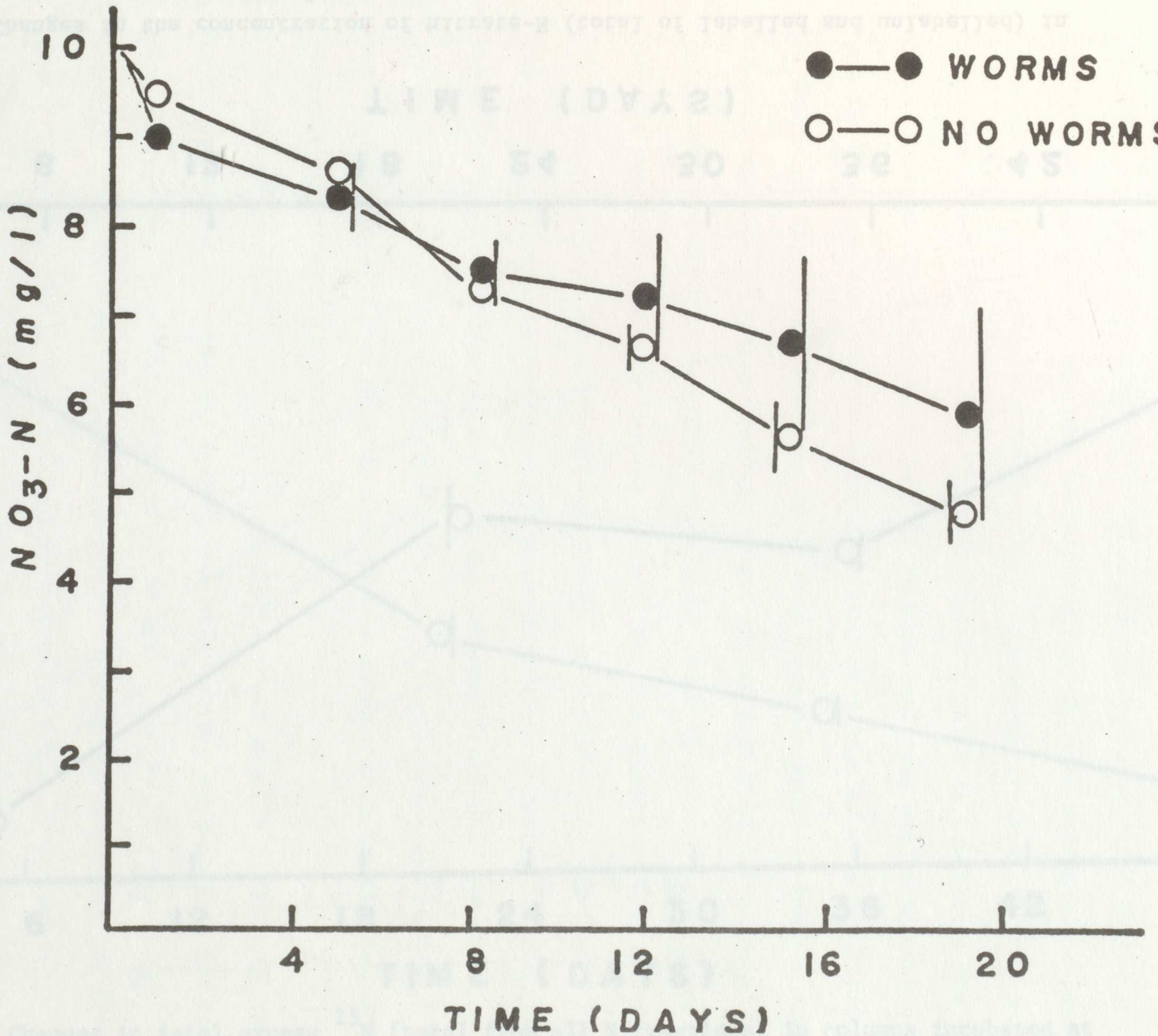

Figure 8: Changes in nitrate-N concentration in the nitrate solution overlying 10 cm of Canagagigue sediment when fresh nitrate was added. Mean of 3 replicates ±SD.

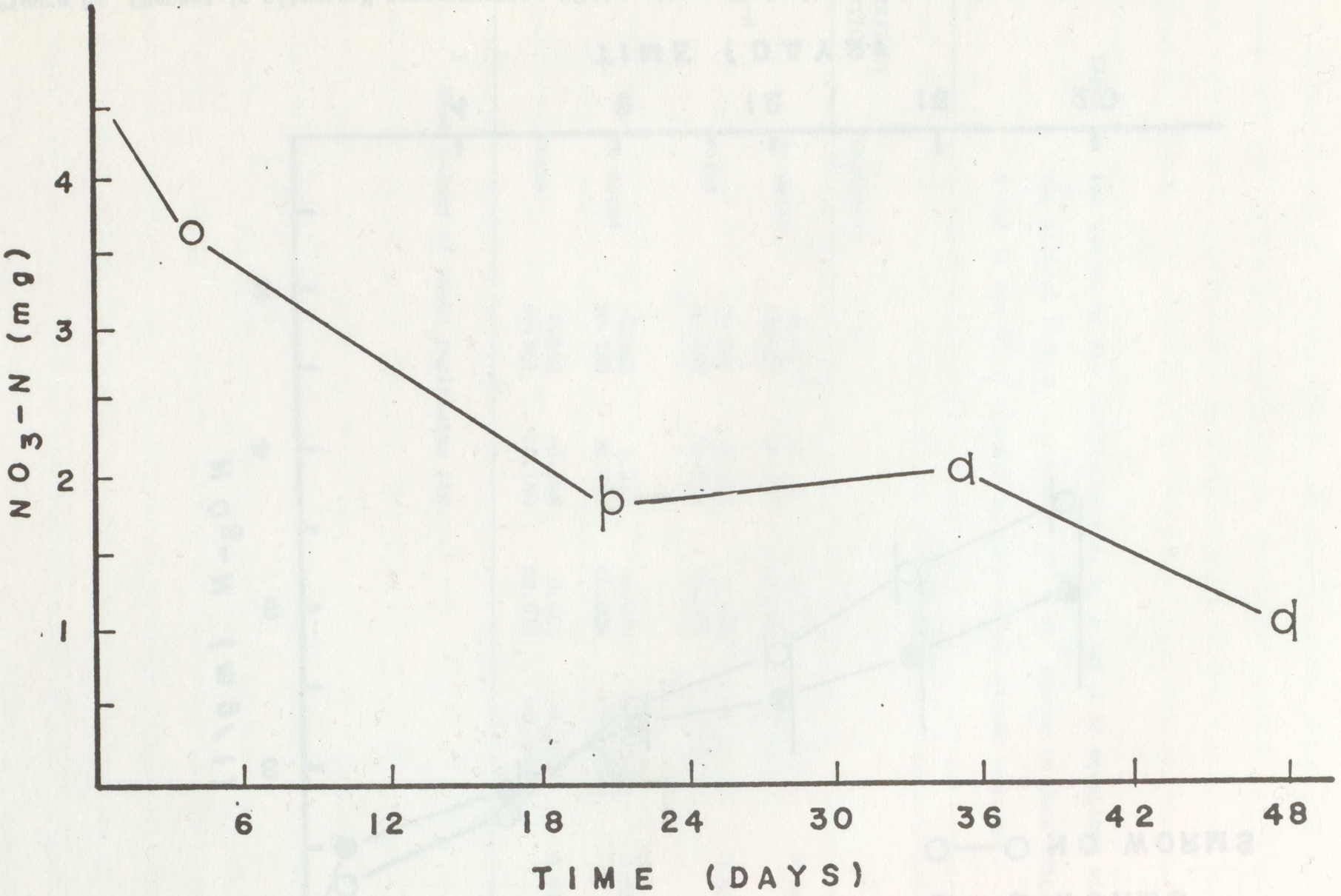

Figure 9: Changes in the concentration of nitrate-N (total of labelled and unlabelled) in columns incubated at 15°C for 48 days. Mean ±SD.

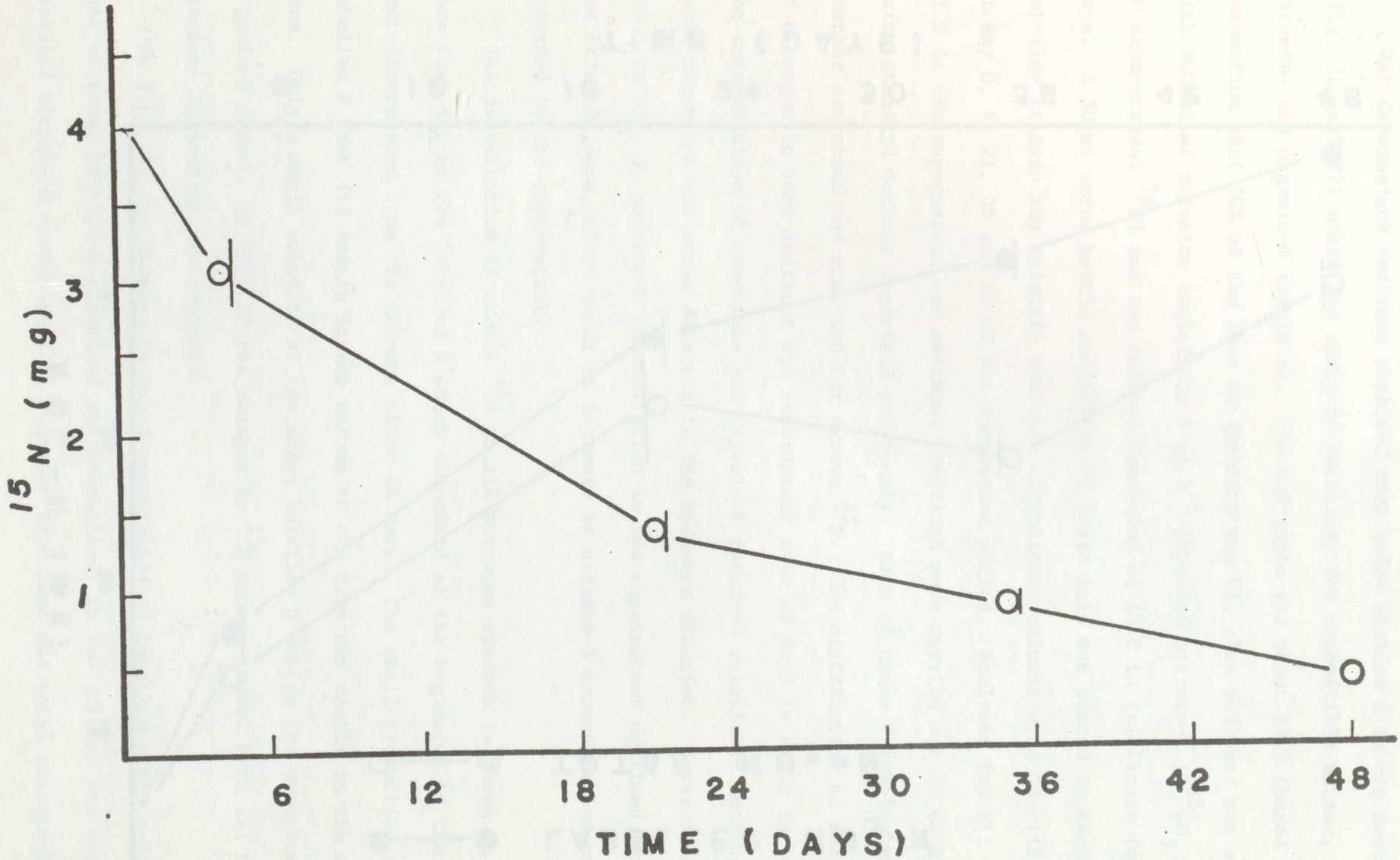

Figure 10: Changes in total excess ^{15}N (total from all N fractions) in columns incubated at 15°C for 48 days. Mean ±SD.

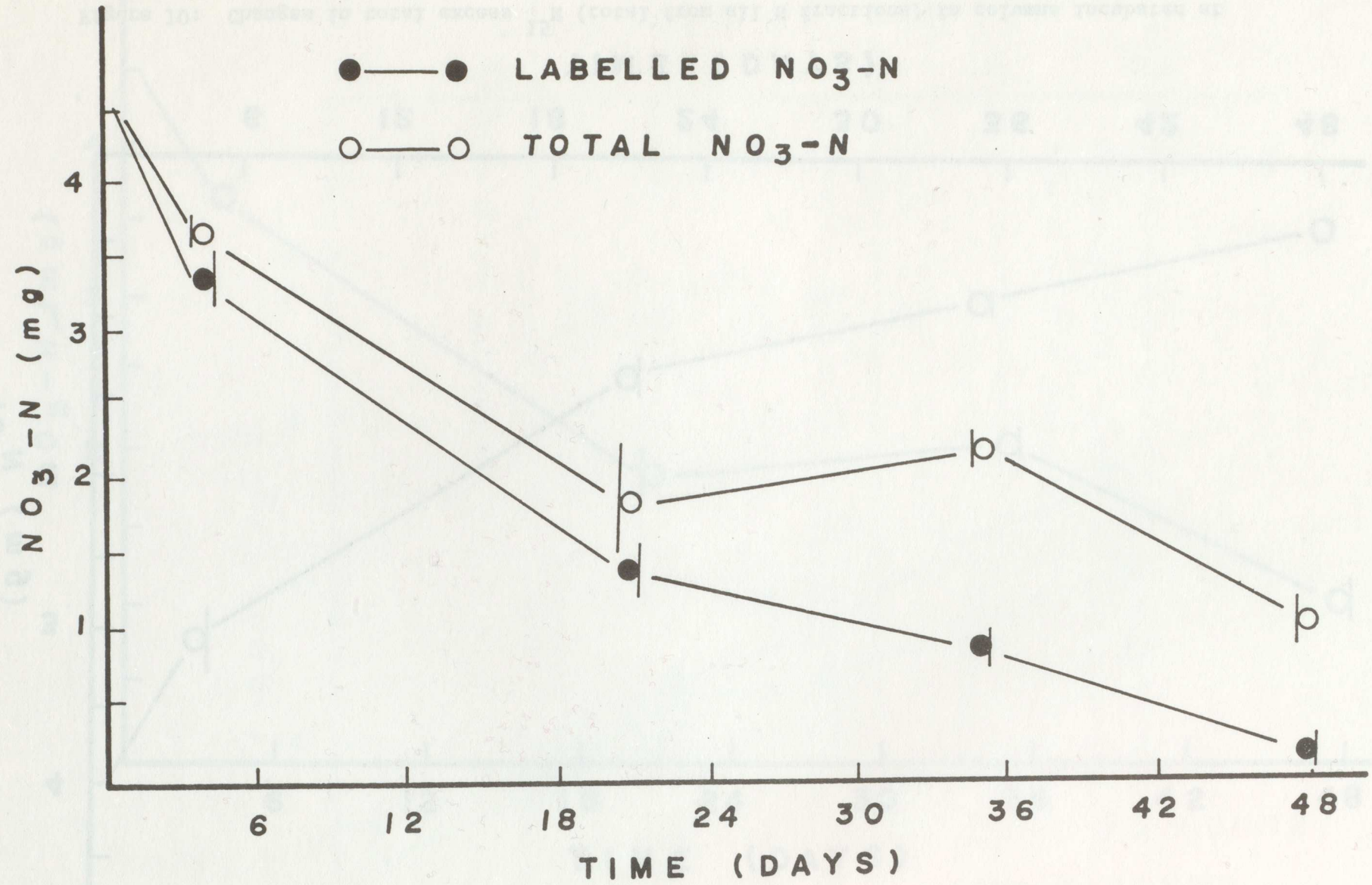

Figure 11: Changes in labelled nitrate-N and total nitrate-N in Canagagigue columns incubated at 15°C for 48 days. Mean ±SD.

Wet Canagagigue sediment obtained from below station 3 in the East branch (Fig. 3) was well mixed and analysed initially for combustible matter, ammonium-, nitrate- and organic-N (Table 4). The C:N ratio was about 13:1 (based on the assumption that 50% of the loss on ignition was C). The sediment was overlain with 500 ml of solution containing 9 mg l^{-1} labelled nitrate-N ($K^{15}NO_3$ with 90 atom-percent ^{15}N) and the columns incubated at 15°C in triplicate for 48 days. A 50-ml serum bottle containing 2% boric acid was placed at each gas out-flow to trap any volatile ammonia. Triplicate columns were "sacrificed" at day 0, 4, 21, 35 and 48 of the incubation period. Analyses for all forms of N in the supernatant and sediment fractions were carried out at each time using standard methods described previously. Each of these N samples was then further processed and analysed for excess ^{15}N. The distribution of all forms of nitrogen in both sediment and supernatant over 48 days is shown in Table 5. The concentration of ammonium- and organic-N remained relatively unchanged while nitrate-N was never detected in the sediment fraction. However, as shown in Fig. 9, nitrate-N concentration in the supernatant declined for the first 21 days, after which an increase in nitrate-N concentration was recorded in the supernatant.

The distribution of excess ^{15}N in all nitrogen species is shown in Table 6. More than 96% of the labelled N which was added at the beginning of the experiment disappeared from the columns after 48 days. The small proportion of labelled N that did remain in the column at any time was mostly in the nitrate-N form. Only a small quantity of the added labelled N was in the ammonium- and organic-N forms. In Fig. 10 the changes in ^{15}N excess (total from all N species) in columns are compared.

In Fig. 11, the changes in the concentration of labelled nitrate-N and that of total nitrate-N (labelled and unlabelled) in the columns are compared. Labelled nitrate-N disappeared at a faster rate than did total nitrate-N. This

TABLE 4: Characterization of Canagagigue sediment used in the ^{15}N experiment.

1.	pH	7.2
2.	NH_4-N (ppm)	47.5
3.	NO_3-N (ppm)	0
4.	Org-N (%)	0.24
5.	Combustible matter (%)	6.4
6.	C:N	13:1

led to a change in ^{15}N abundance from about 90 atom-percent initially to about 15 atom-percent at the end of the experiment. This indicated that substantial nitrate production occurred in the columns as a result of nitrification. By using the known level of isotope dilution at each sampling time, the quantity of nitrate-N produced in the columns was calculated from the following derived equation:

$$N_g = N_1 - N_0 \left(\frac{A_0N_0 - A_1N_1}{\frac{A_0 + A_1}{2}} \right)$$

where: N_g = quantity of N gained through nitrification

A_0 = the atom-percent excess ^{15}N in the initial fraction

A_1 = the atom-percent excess ^{15}N in the final fraction

N_1 = the quantity of N in the final fraction

On this basis, the changes in nitrate production, shown in Fig. 12, were obtained. The rate of nitrification over the 49 days of the experiment and extrapolating to the stream was calculated at 29 mg m^{-2} of stream sediment surface day^{-1}.

Table 5 shows that the amount of labelled N found in the ammonium-N form at any sampling time was very small. This was also true with respect to organic-N. At maximum concentration, on day 21, labelled ammonium-

TABLE 5: The distribution of NH_4-N, NO_3-N and Organic-N in the supernatant and sediment fractions of the columns during the 48-day incubation period at 15°C. Mean of 3 replicates ±SD (mg).

Column Fraction	Nitrogen Species	Incubation Period (Days) 0	4	21	35	48
Supernatant	NH_4-N	0	.49 ±.12	.52 ±.40	0	0
	NO_3-N	4.55 ±.05	3.64 ±.07	1.79 ±.41	2.14 ±.16	1.04 ±.17
	Org-N	0	.21 ±.01	.39 ±.16	.29 ±.04	.49 ±.21
Sediment	NH_4-N	7.70 ±.10	7.68 ±.22	8.40 ±.55	8.25 ±.32	8.23 ±.29
	NO_3-N	0	0	0	0	0
	Org-N (Soluble)	.76 ±.03	.80 ±.08	.66 ±.12	.60 ±.20	1.25 ±.03
	Org-N (%)	.245 ±.005	.240 ±.006	.233 ±.008	.228 ±.014	.228 ±.012

TABLE 6: The distribution of excess ^{15}N NH_4-N, NO_3-N and Organic-N in supernatant and sediment fractions of the columns during the 48-day incubation period at 15°C. Means of 3 replicates ±SD (mg x 10^2).

Column Fraction	Nitrogen Species	Incubation Period (Days) 0	4	21	35	48
Supernatant	NH_4-N	0	1.03 ±.19	1.47 ±1.24	0	0
	NO_3-N	408.64	302.80 ±24.53	126.01 ±11.85	75.85 ±6.63	13.41 ±2.86
	Org-N	0	1.18 ±.05	3.11 ±1.29	.60 ±.17	1.37 ±.58
Sediment	NH_4-N	0	2.27 ±.42	4.74 ±.52	3.30 ±1.14	0
	NO_3-N	0	0	0	0	0
	Org-N (Soluble)	0	0.48 ±.08	0.38 ±.16	0.48 ±.04	0.36 ±.06
	Org-N	0	0	0	0	0
	TOTAL	408.64	307.75 ±24.27	135.71 ±8.95	83.23 ±6.54	15.14 ±2.71

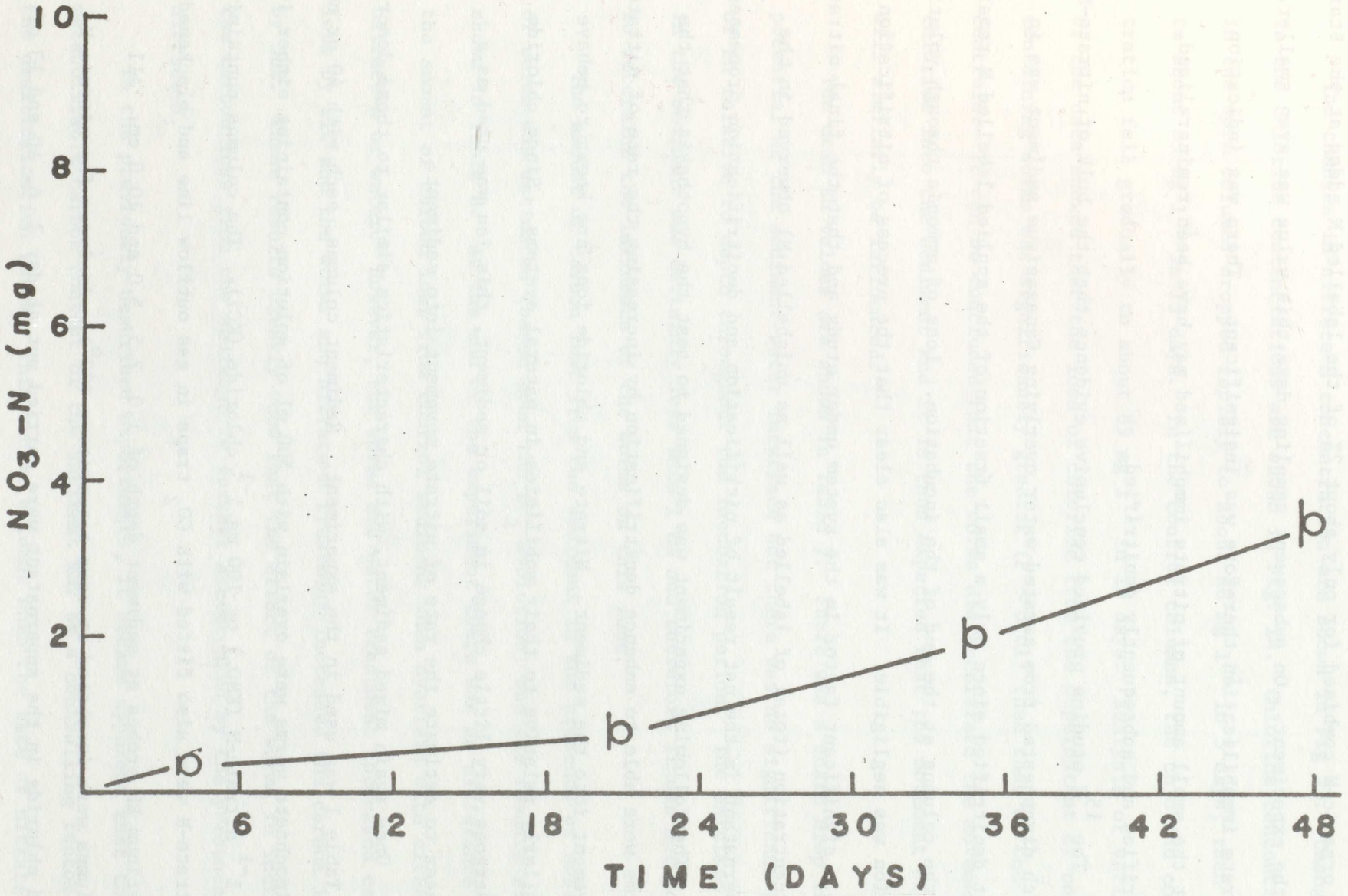

Figure 12: Estimated production of unlabelled nitrate-N in sediment columns incubated at 15°C for 48 days. Mean ±SD.

and organic-N combined for only about 2% of the labelled N added at the start of the experiment. On subsequent sampling days this value was even smaller. Nitrate immobilization therefore was insignificant. There was indication that the small amount of nitrate immobilized may have been remineralized, nitrified and subsequently denitrified.

The ^{15}N studies provided conclusive evidence that the bulk of nitrate-N which disappeared from aerated water overlying Canagagigue sediment was in fact denitrified since only a small fraction of the applied labelled N remained in the columns at the end of the incubation. Loss of ammonia through volatilization was negligible. It was also clear that the process of nitrification was a significant factor in the system under study and that the final nitrate-N concentration (total of labelled as well as unlabelled N) observed in the supernatant is the net result of nitrification and denitrification processes.

The following experiment was designed to test the hypothesis that the worms were able to enhance denitrification by increasing the rate of nitrate movement into the sediment. Nitrate and chloride ions are known to behave similarly relative to their mobilities in natural systems. Since chloride undergoes very little change in soil or sediment, this ion was used as a tracer to estimate the rate of nitrate movement into sediment.

Once again mixed sediment, with characteristics similar to those described in Table 1 was used in the experiment. Sediment columns, each with 40 or no oligochaete worms were overlain with 500 ml of solution containing either 10 mg l^{-1} nitrate-N (KNO_3) or 100 mg l^{-1} chloride (KCl). The columns containing nitrate-N were also fitted with CO_2 traps in gas outflow line and blackened platinum Eh probes at sediment depth of 1.0, 2.5, 5.0 and 10.0 cm. All columns were incubated at room temperature (22^{o}). Analyses for nitrate-N and chloride in the supernatant were carried out at day 3, 6, 10 and 13 of incubation. CO_2 evolution and Eh readings were also recorded at each time.

The changes in nitrate-N concentration are shown in Fig 13. Changes in the chloride concentrations are shown in Fig. 14. Chloride concentrations in the worm columns dropped rapidly to about 88 mg l^{-1} in 3 days, then slowed to about 85 mg l^{-1} in 13 days. In the control columns, the chloride concentration fell gradually to about 89 mg l^{-1} in 13 days. Analysis of variance of the data showed that the loss of chloride from the supernatant of columns containing worms was significantly greater ($P < 0.01$) than that of the controls. It can be concluded that the worms did accelerate the movement of chloride into the sediment and it is thus expected that the movement of nitrate will similarly be influenced.

The mean Eh values are shown in Table 7. There was a sharp drop in redox potential at the sediment-water interface but there was no significant shift of Eh relative to depth throughout the incubation period. The oxidised zone in the sediment was certainly less than 1 cm and therefore it was not possible to determine the influence of the worms on this zone.

The amount of carbon lost from the sediment in the presence or absence of worms was estimated (based upon the CO_2 evolution) and the results are shown in Table 8. At the end of the experiment there was no difference in the amount of carbon lost from control sediment when overlain with either nitrate-N or water. However, the worms and water treatment increased carbon loss by 21% and the worms and nitrate-N treatment increased the carbon loss by 58%. These results clearly show that the effect of the worms in increasing the carbon loss from the sediment is further accentuated by the presence of nitrate-N.

In the previously-described experiments, it became apparent that the metabolizable carbon content of the sediment may be a controlling factor in the denitrification rates. This became evident when the rate of nitrate loss from water overlying Canagagigue sediment decreased upon repeated additions

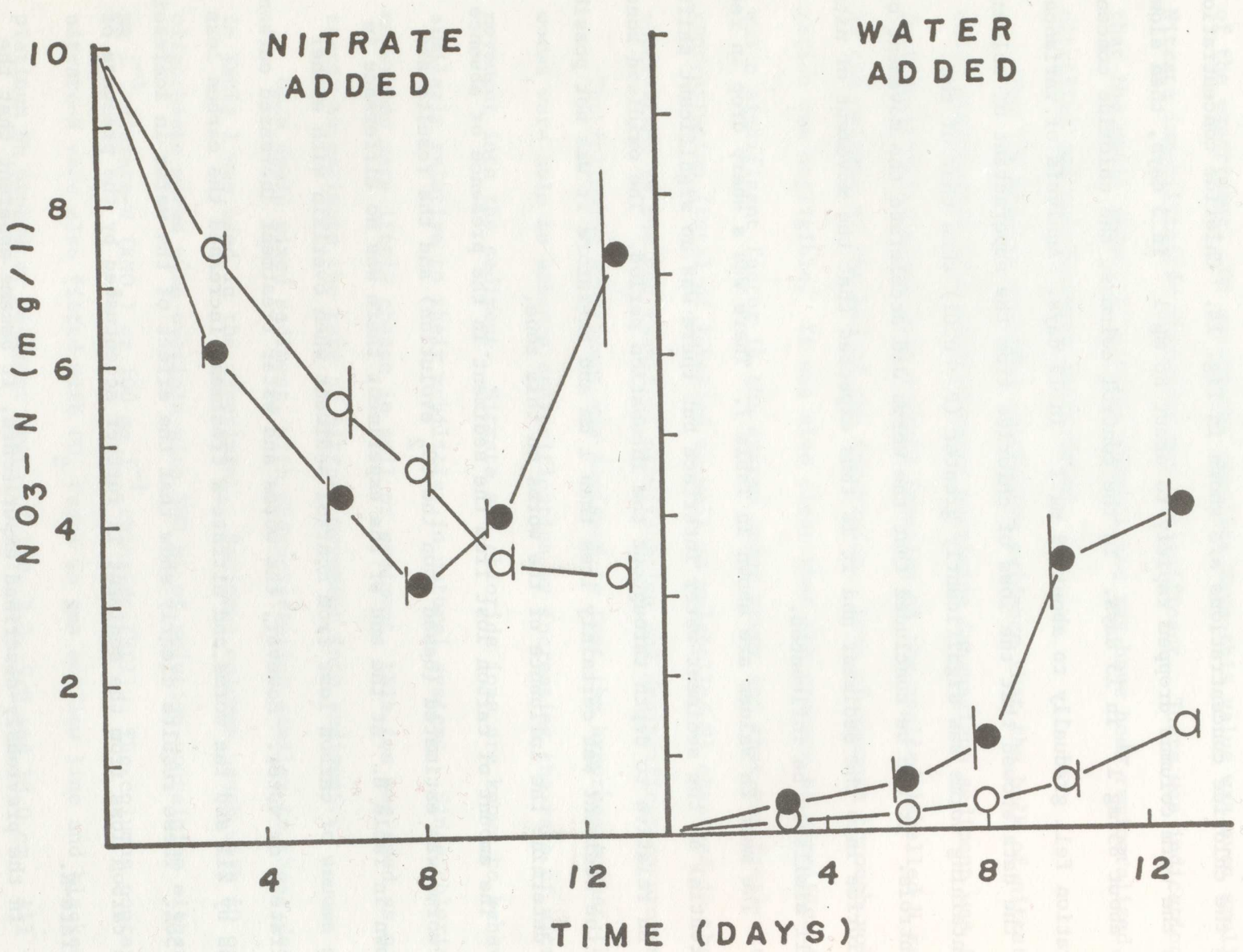

Figure 13: Changes in nitrate concentrations in Canagagigue columns incubated at 22°C for 13 days with (● —— ●) without (O —— O) oligochaete worms. Mean ±SD.

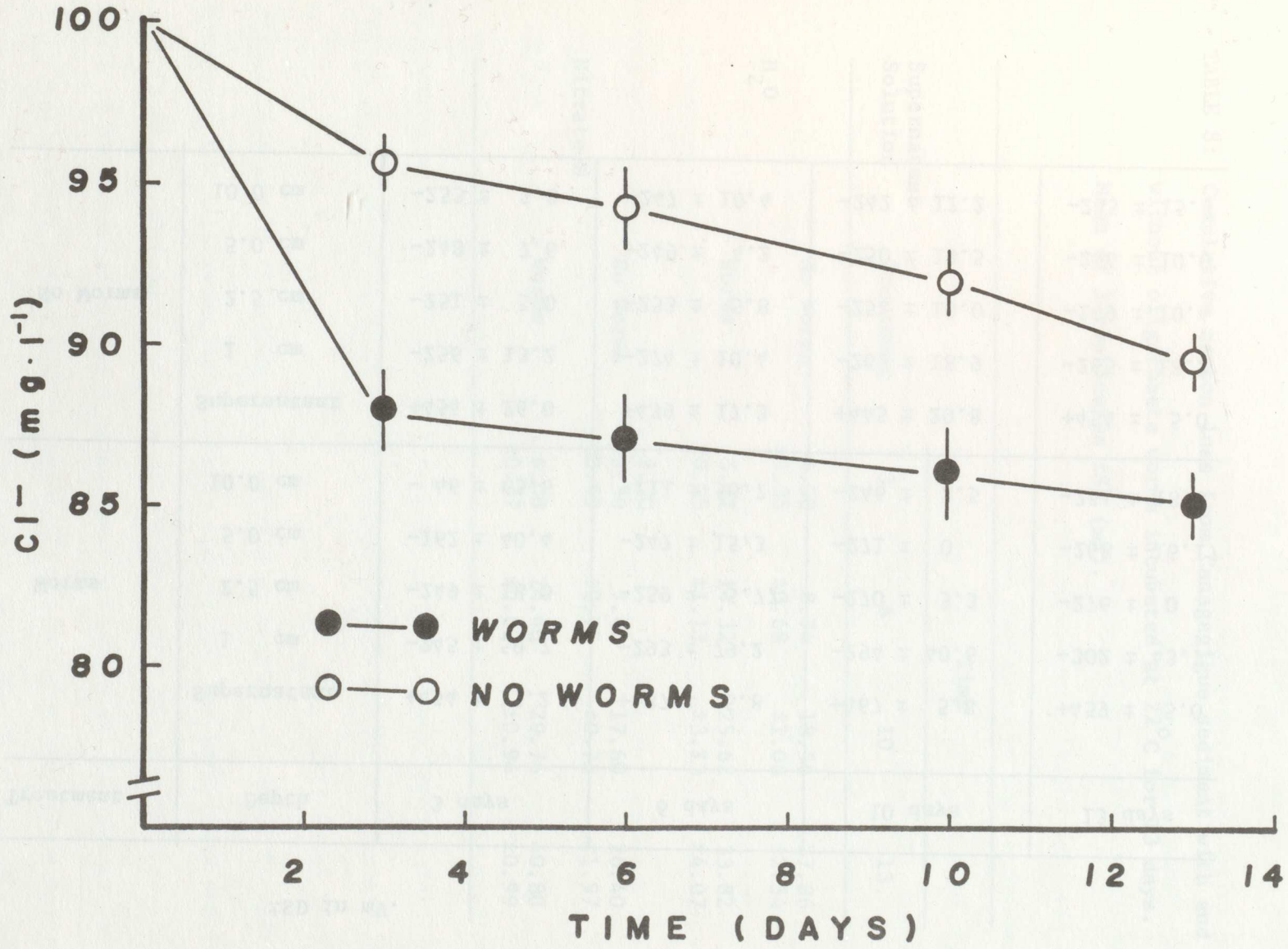

Figure 14: Changes in chloride concentrations in Canagagigue columns overlain with 100 mg l^{-1} KCl and incubated at 22°C for 13 days with (● —— ●) and without (○ —— ○) oligochaete worms. Mean ±SD.

TABLE 7: Changes in redox potential at different depths of Canagagigue sediment with and without oligochaete worms. Mean of 3 replicates ±SD in mV.

Treatment	Depth	3 days	6 days	10 days	13 days
Worms	Supernatant	+454 ± 13.2	+437 ± 5.8	+467 ± 5.8	+459 ± 5.0
	1 cm	-245 ± 59.7	-293 ± 79.2	-294 ± 40.6	-302 ± 43.7
	2.5 cm	-249 ± 18.0	-259 ± 5.77	-270 ± 5.3	-276 ± 0
	5.0 cm	-162 ± 40.4	-247 ± 15.3	-271 ± 0	-268 ± 16.17
	10.0 cm	- 46 ± 65.6	-211 ± 50.7	-246 ± 2.5	-251 ± 18.0
No Worms	Supernatant	+454 ± 26.0	+439 ± 17.3	+445 ± 20.8	+454 ± 5.0
	1 cm	-256 ± 13.2	-274 ± 10.4	-269 ± 18.9	-265 ± 18.2
	2.5 cm	-251 ± 5.0	-253 ± 5.8	-252 ± 10.0	-249 ± 10.4
	5.0 cm	-248 ± 7.6	-249 ± 4.2	-250 ± 13.5	-246 ± 10.0
	10.0 cm	-253 ± 5.8	-247 ± 10.4	-242 ± 12.2	-245 ± 15.3

TABLE 8: Cumulative carbon loss from Canagagigue sediment with and without oligochaete worms incubated at 22°C for 13 days. Mean of 3 replicates ±SD (mg).

Supernatant Solution	Treatment	Time 3	6	10	13
H_2O	No Worms	4.00 ±0.35	8.74 ±0.68	18.56 ±1.08	27.86 ±2.54
	Worms	5.72 ±0.25	14.32 ±1.13	25.62 ±3.13	33.82 ±4.07
Nitrate-N	No Worms	2.30 2.30 ±0.69	7.40 ±0.75	17.60 ±0.75	26.40 ±1.97
	Worms	6.08 ±0.37	14.46 ±0.63	29.76 ±0.94	40.80 ±0.99

of fresh nitrate solution to the columns.

One experiment was designed to determine whether or not the organic matter content of Canagagigue sediment had any influence on denitrification rates. The sediment was analysed at the beginning of the experiment for ammonium-, nitrate- and organic-N and for combustible matter. Table 9 shows that the organic matter content was fairly substantial (10-25%) and the concentrations of ammonium- and organic-N were relatively high. Nitrate-N was not detected. The thoroughly mixed wet sediment was used to pack columns as before. In addition, triplicate columns received sediment mixed with varying amounts of organic matter in the form of dried ground leaves (3:1 mixture of maple, Acer saccharum and water-cress, Nasturtium officinale). Each treatment thus received 0, 1, 5 and 10 percent (w/w, air-dry basis) leaf material. Also sediment to which nitrate was added in two successive runs was supplemented with 1% of the leaf material and included in the experiment. All the columns were topped with 10 mg l^{-1} nitrate solution (KNO_3), aerated with 21% oxygen in helium and incubated at room temperature ($22^{o}C$). To estimate the carbon oxidation rates, the gas outflow of each column was fitted with carbon dioxide traps. Nitrate-N concentration in the supernatant was determined and carbon dioxide evolution was measured at day 0, 3, 6, 10 and 13 of incubation.

Fig. 15 shows the changes in nitrate-N concentration in the supernatant of columns containing varying amounts of added leaf material. The nitrate-N concentration in the controls dropped gradually to about 3 mg l^{-1} in 13 days. However, almost all the nitrate-N disappeared from the columns with 1, 5 and 10 percent added leaf material in 12, 10 and 8 days respectively. The nitrate-N loss (not shown) from columns with the "spent" sediment + 1% ground leaves was in the same order of magnitude as that of the 1% leaf treatment. Analyses of variance of the data show that the differences in

TABLE 9: Characterization of the Canagagigue sediment used to determine the effects of ground leaves on denitrification rates. Mean of 3 replicates ±SD.

1.	pH	7.3
2.	NH_4-N (ppm)	56.0 ± 3.8
3.	NO_3-N (ppm)	0
4.	Org-N (%)	0.32 ± 0.02
5.	Combustible matter (%)	10.25 ± 0.30
6.	C:N	16:1

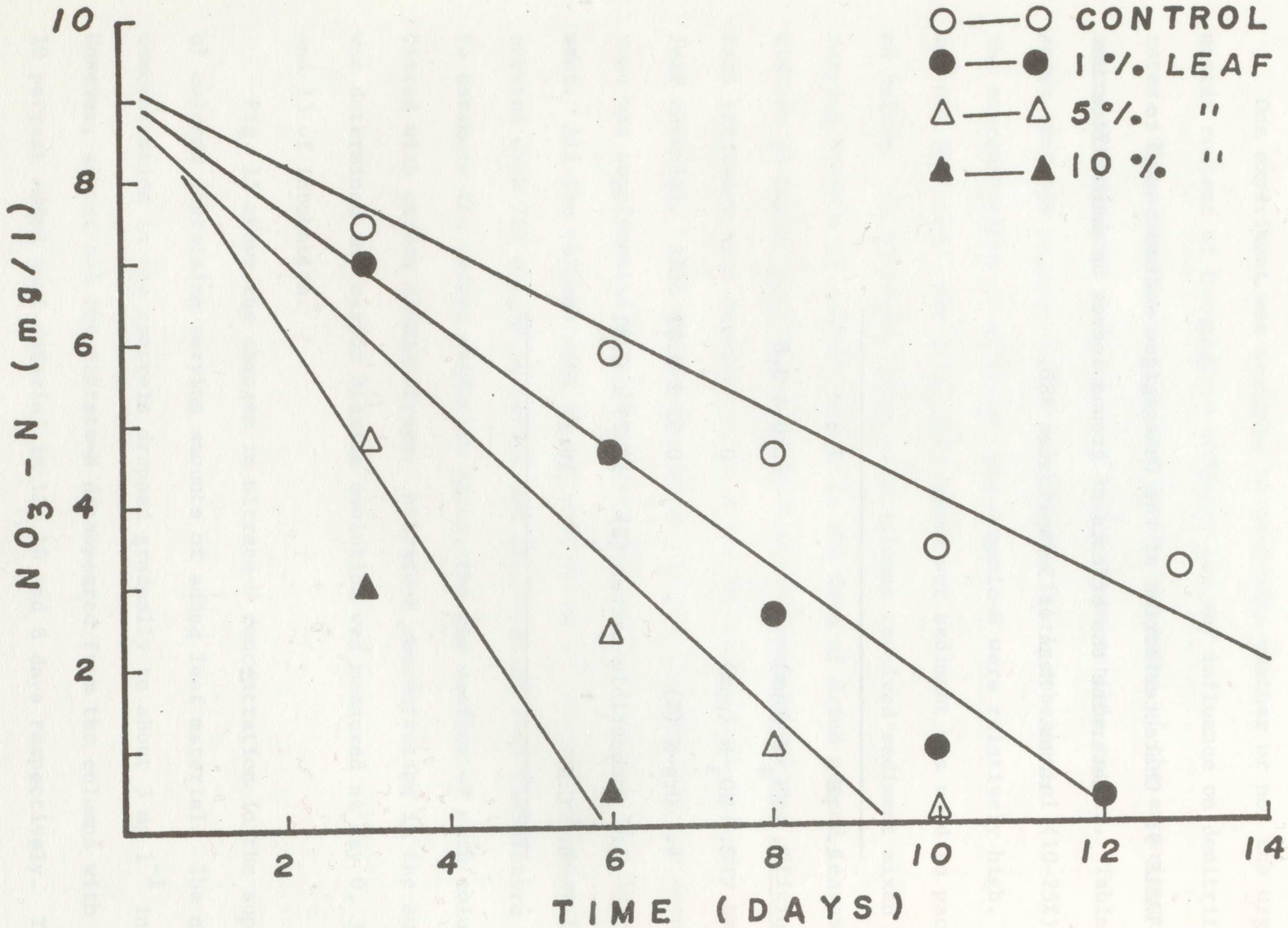

Figure 15: Change in nitrate-N concentration in Canagagigue columns following additions of varying amounts of a mixture of maple and water-cress leaves. Mean of 3 replicates with fitted linear regression lines.

the loss of nitrate due to the added leaf material were significant ($P < 0.01$). From these results, it appears that an increase in the carbon content of sediments will also increase the denitrification rate although the possibility of other processes (such as immobilization) cannot be excluded.

The accumulated carbon loss, based on CO_2 evolution, is shown in Fig. 16. At the end of the experiment the control columns had lost an average of about 25 mg carbon. The corresponding values for the 1, 5 and 10 percent leaf treatments were 32, 64 and 95 mg of carbon. Analysis of variance of the data showed that the differences in the carbon loss due to the leaf treatments were also significant ($P < 0.01$). It seems reasonable to conclude that the addition of available organic matter to Canagagigue sediment significantly enhances denitrification. This is likely the result of the additional energy source provided by the ground leaves as evidenced by the increased respiration rate.

Observations on nitrate loss from water overlying sediment from two other PLUARG streams, Holiday Creek (Ag 5) and Little Ausable River (Ag 3) were made. Sediment samples which were taken from three locations in each stream varied widely in organic matter content (Table 10). Sediment columns were set up as described before and incubated at room temperature. Nitrate-N changes in the supernatant of columns containing Holiday Creek sediment are shown in Fig. 17. About 75% of the added nitrate-N disappeared from the overlying water in two weeks. The rates of nitrate-N losses were similar for sediments from each of the sample sites. Similar observations were made when sediment from Little Ausable River was used (Rig. 18).

11.2 Field Studies

In 1975 and 1976 nitrogen and chloride concentrations in the two branches of upper Canagagigue Creek were monitored in order to select a reach for detailed study of denitrification. Table 11 is an example of the data and summarizes the period between September 1975 and March 1976. Highly concen-

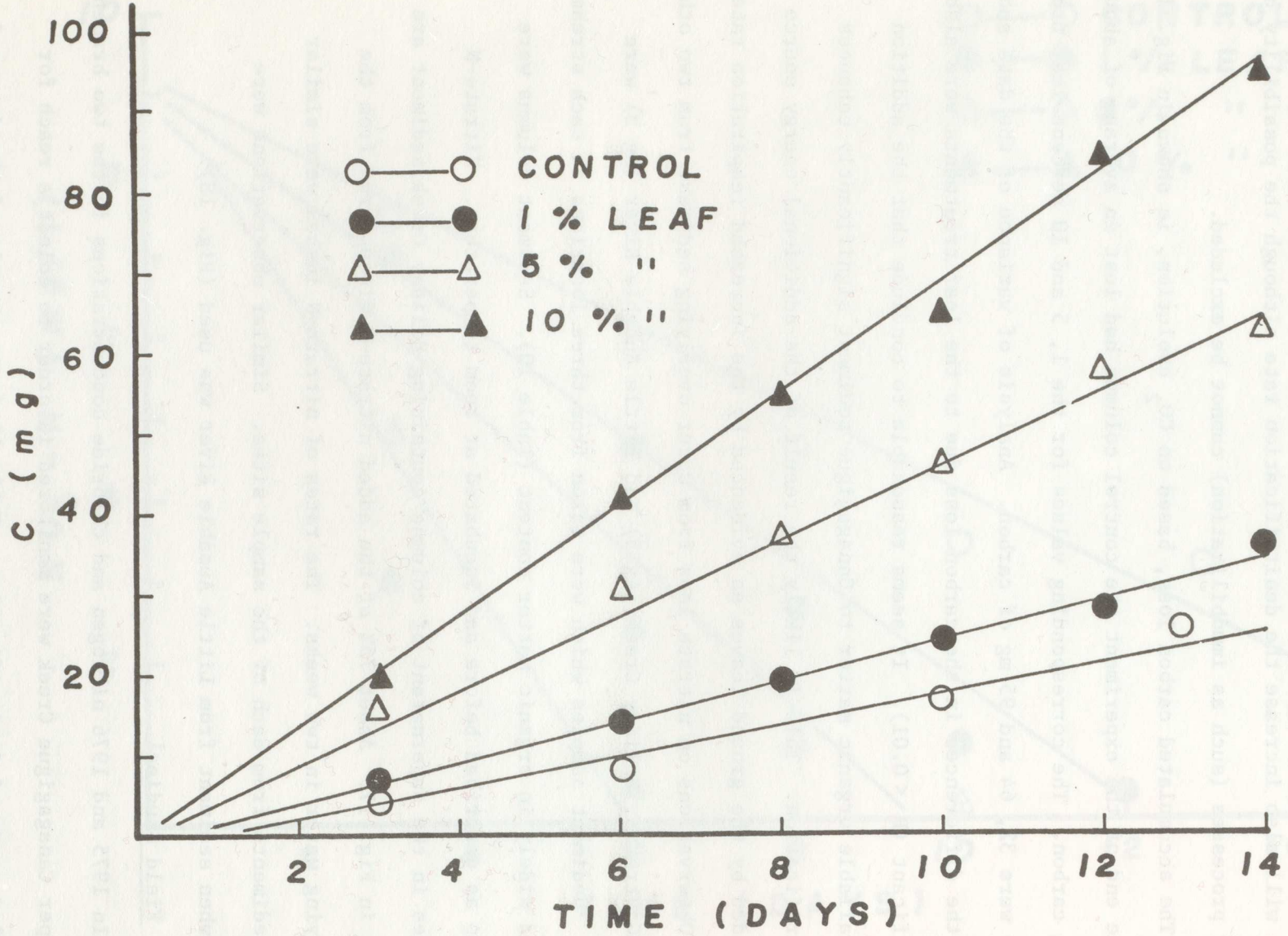

Figure 16: Accumulated carbon loss from Canagagigue columns following additions of varying amounts of a mixture of maple and watercress leaves. Mean of 3 replicates with fitted linear regression lines.

TABLE 10: Combustible matter content (%) of sediments from Holiday Creek and Little Ausable River.

Sites	Holiday Creek	Little Ausable River
1	2.27 ± 0.21	4.03 ± 0.37
2	5.37 ± 0.30	2.53 ± 0.19
3	7.33 ± 0.20	2.21 ± 0.17

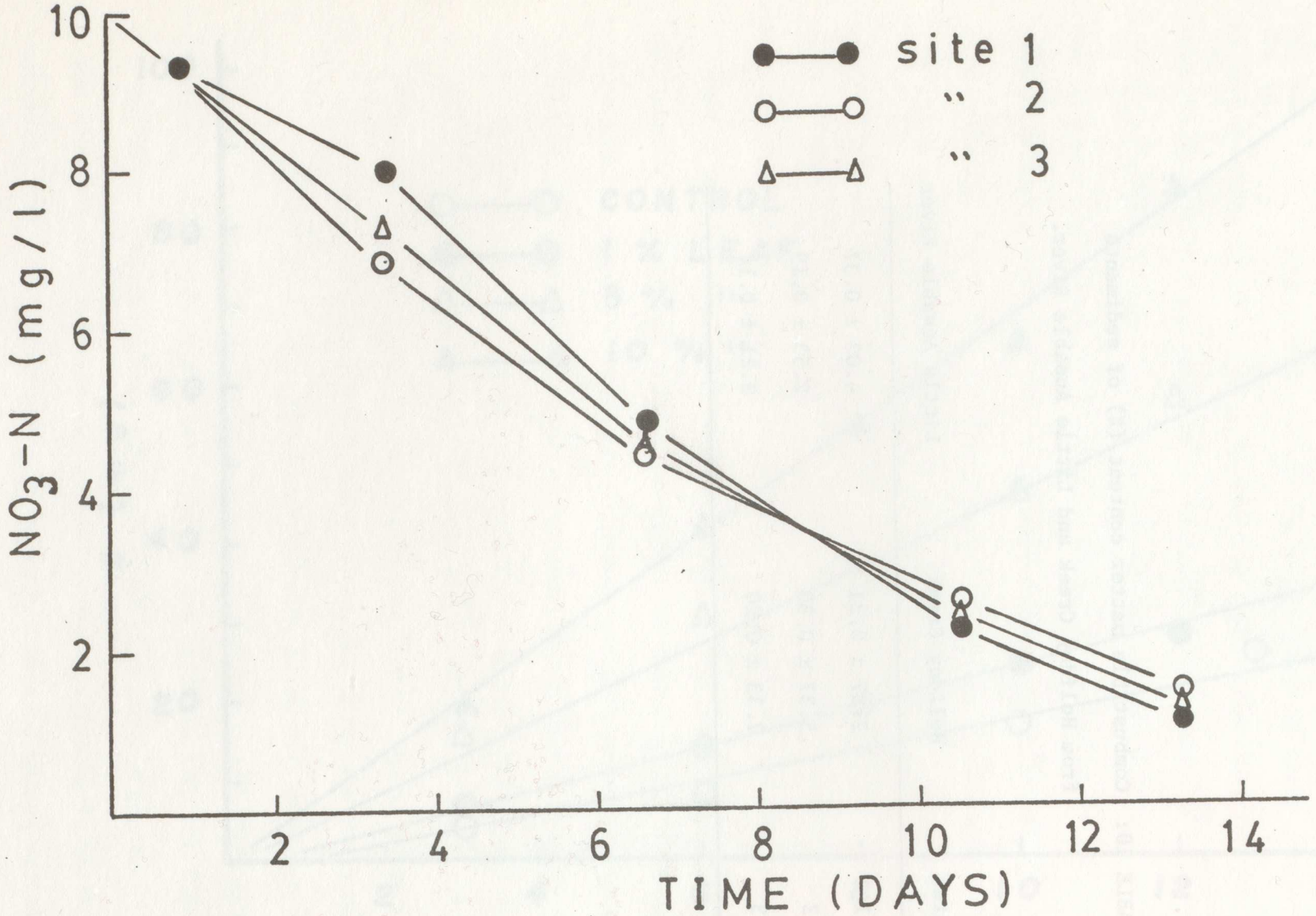

Figure 17: Changes in nitrate-N concentration in water overlying sediment from 3 sites of Holiday Creek. Means of three replicates.

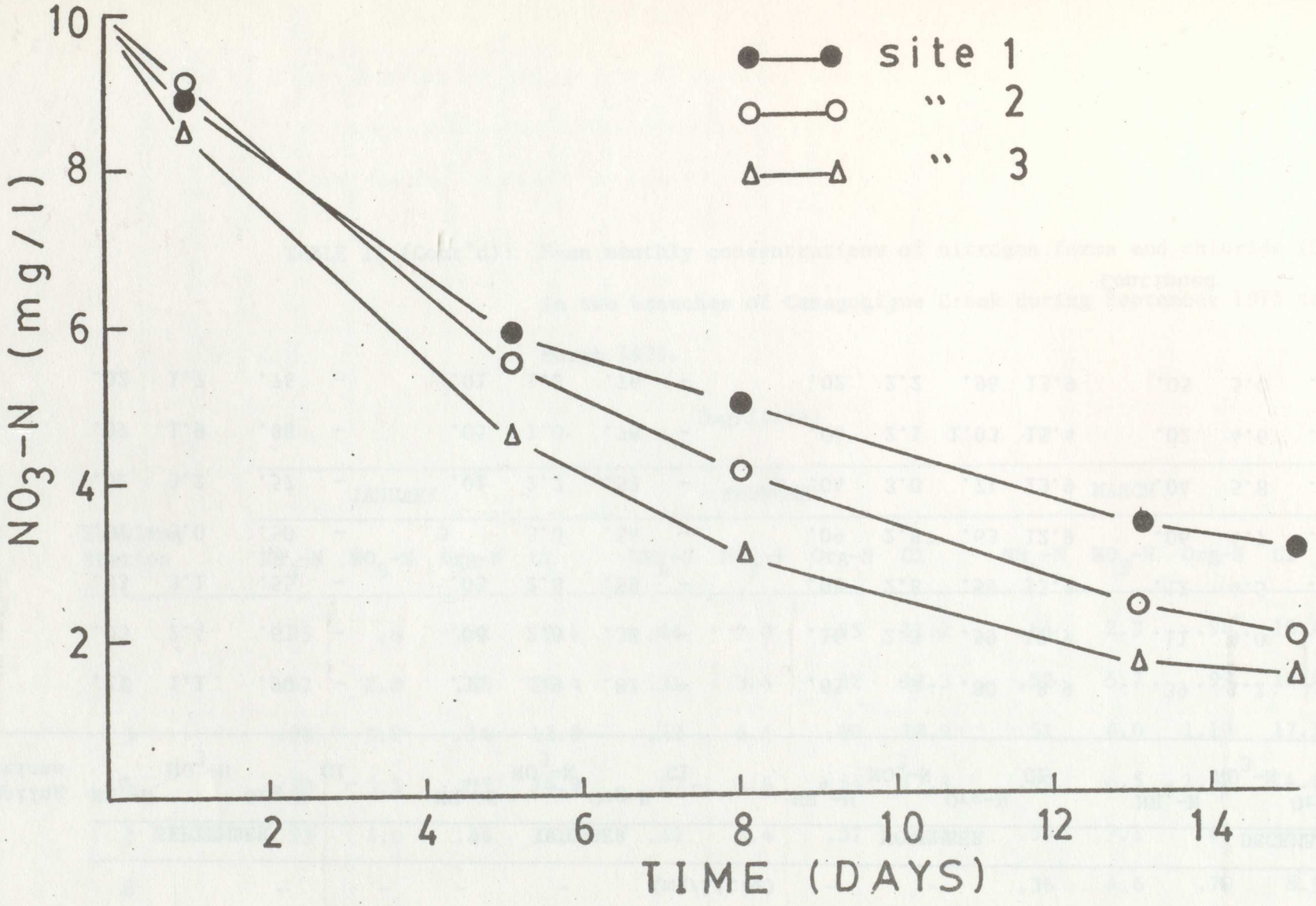

Figure 18: Changes in nitrate-N concentration in water overlying sediment from 3 sites of Little Ausable River. Means of three replicates.

TABLE 11: Mean monthly concentrations of nitrogen forms and chloride in two branches of Canagagigue Creek during September, 1975 to March, 1976.

(mg/litre)

Sampling Stations	SEPTEMBER				OCTOBER				NOVEMBER				DECEMBER			
	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-
1	.21	1.1	.90	-	.35	.9	.82	-	.65	.9	.80	8.9	.39	1.2	.41	9.8
2	.03	2.4	.61	-	.06	2.3	.38	-	.16	2.3	.59	12.9	.11	4.0	.38	17.2
3	.03	3.1	.55	-	.03	2.8	.59	-	.08	2.8	.59	13.9	.12	4.5	.44	17.2
5	.02	3.0	.50	-	0	3.0	.54	-	.09	2.8	.63	12.9	.06	4.7	.39	16.6
7	.02	3.2	.57	-	.01	2.7	.52	-	.04	3.0	.71	13.9	.04	5.8	.42	16.7
8	.07	1.9	.88	-	.05	1.0	.78	-	.04	2.1	1.03	15.4	.02	4.6	.72	12.7
9	.02	1.7	.76	-	.01	1.2	.76	-	.02	2.2	.96	13.9	.05	5.0	.70	12.9

Continued

TABLE 11 (Cont'd): Mean monthly coneentrations of nitrogen forms and chloride (Cl^-) in two branches of Canagagigue Creek during September 1975 to March 1976.

(mg/litre)

Sampling Station	JANUARY				FEBRUARY				MARCH			
	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-
1	.75	.8	.52	9.4	.64	1.0	.55	11.2	.64	2.3	.94	10.4
2	.32	2.8	.58	16.4	.31	3.4	.62	17.3	.52	5.7	.99	18.4
3	.29	3.2	.54	13.9	.43	4.4	.60	18.9	.51	6.0	1.10	17.1
5	.20	3.5	.35	14.9	.27	4.6	.54	17.9	.40	6.5	1.02	14.6
7	.23	4.0	.46	16.2	.42	5.4	.57	15.1	.59	7.1	.68	14.6
8	-	-	-	-	-	-	-	-	.36	4.6	.70	8.9
9	.16	3.2	.59	10.7	-	-	-	-	.49	5.8	.68	18.9

trated additions of nitrate-N were made to the East branch by continuously flowing tiles which discharge into the stream (Table 12). A reach between stations 3 and 7 on the East branch was selected for intensive studies. In the spring of 1976 a staff gauge was placed at station 3 and detailed monitoring was carried out twice per week over a period from July to October 1976, when flow was at seasonal low.

A budget was prepared for the reach by summing QXC for station 3 and each of the inputs (tiles, tributaries and ground water) and comparing to QXC at station 7. The flow at station 7 which could not be accounted for by flow at station 3 or tile input or tributary input was assumed to be groundwater input at a nitrate-N concentration of 5 ppm. Calculations made for each of the days of record for 1976 showed extreme variation (Table 13). Out of the 18 sampling days during this period apparent losses ranged from 1.1% to 18.8% of the nitrate-N input while on one day there appeared to be a gain in nitrate-N over the reach. A further attempt at budgeting nitrogen was made during spring of 1977. The staff-gauge was abandoned in favour of gauging with an Ott meter. However, it became apparent that gauging at station 3 was so imprecise that it was impossible to establish consistent trends for gains, losses or a balance in the nitrogen budget.

On November 2, 1977, a concerted effort was made to survey the entire reach between stations 3 and 7 once more. Gauging by Ott meter and sampling were carried out at several sites along the main stream. The same was done for each tile and surface inflow. Nitrate additions through ground water were estimated on the basis of increased flow rate at the downstream site and the mean concentration of nitrate concentration in the ground water seeps adjacent to the stream. We found that between stations 3 and 5, the nitrate-N loss was 32 mg/sec and between stations 5 and 7, it was 23.5 mg/sec.

TABLE 12: Mean monthly concentrations of nitrogen forms and chloride (Cl^-) in the continuously-flowing tiles discharging into the East Branch, Canagagigue Creek during September to December 1975.

Sampling Stations	SEPTEMBER				OCTOBER				NOVEMBER				DECEMBER			
	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-	NH_4-N	NO_3-N	Org-N	Cl^-
T7	0	15.5	.09	-	0	15.8	.05	-	0	12.2	.55	13.9	0	13.3	.26	17.7
T8	0	18.6	.09	-	0	20.7	0	-	0	18.2	.09	9.9	-	-	-	-
T9	.01	12.0	.06	-	0	15.0	.04	-	0	12.9	.18	8.9	0	15.0	.13	7.4
T10	.01	14.4	.07	-	0	17.7	.04	-	.02	15.9	.54	10.9	0	16.7	.15	10.4

TABLE 13: Nitrate-N budget for Canagagigue Creek (East branch) between stations 3 and 7 during the period July to October 1976.

DATE	TOTAL INPUT (mg/sec)	TOTAL OUTPUS (mg/sec)	% CHANGE	FLOW AT STATION 7 (litres sec^{-1})
July 15	568.36	561.10	- 1.3	155
19	504.3	520.55	+ 3.2	145
22	595.91	572.25	- 4.0	175
26	541.68	474.15	-12.5	145
29	1038.85	960.0	- 7.6	640
Aug. 5	434.84	417.60	- 4.0	120
16	521.13	478.95	-10.2	155
23	404.5	351.60	-13.1	120
30	499.81	464.26	- 7.1	139
Sept. 2	425.99	421.20	- 1.1	135
9	410.67	367.22	-10.6	122
16	414.01	362.40	-12.5	120
20	470.74	465.12	- 1.2	152
23	963.07	896.04	- 7.0	342
30	464.21	384.25	-17.2	145
Oct. 14	438.23	379.35	-13.4	135
18	434.34	401.70	- 7.5	130
21	725.96	589.50	-18.8	225

For the entire reach studies, nitrate-N loss for that day was calculated at 342.5 $mg/m^2/d$. This represented a loss of 7.4% of the total nitrate-N input.

12.0 Data Interpretation and Conclusions

Laboratory studies using sediment from upper Canagagigue Creek, which drains an area under intensive agriculture, and ^{15}N tracer provided conclusive evidence that the nitrate-N which disappeared from aerated water overlying sediment columns was denitrified since only about 4% of the added labelled nitrate remained in the columns at the end of the experiment. It was also clearly demonstrated in these experiments that significant nitrification occurred and was proceeding concurrently with denitrification. There was evidence that only a small amount (2%) of the labelled nitrate-N was immobilized. The results of these experiments demonstrate that denitrification is likely to be a natural occurrence in streams.

The use of labelled nitrate-N made it possible to follow the sequence of nitrification-denitrification reactions. Redox probes buried in the sediment indicated that despite constant aeration, the aerobic surface layer extended not far below the sediment-water interface. Nitrate-N present in the overlying solution diffuses down to the anaerobic layer where it is denitrified. Meanwhile, ammonium-N originally present in the sediment plus that produced by mineralization diffuses up to the aerobic surface layer where it is nitrified. The nitrate-N thus formed then diffuses into the anaerobic layer where it undergoes denitrification although it first may diffuse into the supernatant water. It is clear from the ^{15}N data that the final nitrate-N concentration observed in the supernatant of the Canagagigue columns is the net result of the two processes, denitrification and nitrification. Therefore, for the determination of real denitrification rates, an estimate of nitrate production must be made.

In this present study, it was shown that denitrification was markedly influenced by temperature: the process proceeding most rapidly at higher

temperatures. Therefore, denitrification in streams during the Summer months is expected to be highest. As in the case of Canagagigue Creek, the depth of water, nature of stream bed and the extent of stream bank vegetation will influence the temperature of the water hence control denitrification rates. Nitrogen losses were significantly correlated with higher temperatures by Sain et al. (1977) and by Van Kessel (1976).

In the present investigation a profound effect of the tubificid oligochaetes on the nitrification and denitrification processes was demonstrated. Column studies using Canagagigue sediment showed that the rates of nitrification and denitrification were significantly greater in the presence of the worms, Tubifex tubifex and Limnodrilus hoffmeisteri. It is believed that the worms are able to enhance the rate of denitrification by increasing the rate of nitrate movement into the sediment. These tube dwellers are known to burrow deep into sediments thereby drawing currents of water to the lower depth. Experiments with chloride solution overlying sediment containing worms supported this theory.

Laboratory studies in which organic material (ground leaves) was added to Canagagigue sediment showed a direct relationship between the amount of added leaf material and denitrification rates. However, as noted before, sediments from the Canagagigue Creek, Swifts Brook, Holiday Creek and Little Ausable River removed nitrate at rates which are in the same order of magnitude although the organic matter in these sediments vary widely. While there is no doubt that denitrification will begin to decline as the level of organic matter (energy) falls below some critical level, it is difficult to make any conclusions without the use of ^{15}N. It is possible that rates of denitrification may be different but concurrent nitrification may be masking such differences.

Laboratory studies carried out on sediments from Canagagigue in this investigation, showed that the net loss of added nitrate-N over the first 12 days at 22°C was about 0.138 g m^{-2} day^{-1}. Experiments conducted similarly with sediment from Holiday Creek and Little Ausable River in Ontario showed net nitrate-N losses similar to that of Canagagigue. In the same time and under similar conditions, sediments from Swifts Brook showed a net loss of about 0.156 g m^{-2} day^{-1}. Van Kessel (1976) reported that sediments from a drainage ditch receiving sewage-plant effluent removed nitrate-N via denitrification at a rate of about 0.175 g m^{-2} day^{-1} under similar experimental conditions as those reported in this present study.

The rate of transport of nitrate into the sediment in nature is expected often to be greater than in laboratory column experiments because diffusional forces such as turbulence and mixing of the sediment by benthic animals are absent in laboratory columns. This assumes, of course, that there is no upward flux of water as would occur at points of groundwater discharge into the stream. Therefore, it is very likely that denitrification in streams and rivers under natural conditions will be higher than those shown under laboratory conditions. It may be for this reason that the field estimate for nitrate loss in Canagagigue was 0.34 g m^{-2} day^{-1}. Similar estimates for the English Rivers, the Great Ouse and the Trent were 0.750 m^{-2} day^{-1} and 1.4 g m^{-2} day^{-1} respectively (Owens et al., 1972). Later, Toms et al. (1975) reported that the denitrification rate in the River Lee in England was between 0.01 to 0.233 g m^{-2} day^{-1}. With such evidence in mind, it is quite conceivable that the N loss for Ontario streams might be greater than 0.2 g m^{-2} day^{-1} where the nitrate concentration exceeds 2 mg l^{-1} and the temperature is near 22°C. At lower concentrations the rate will decrease since nitrate diffusion will decrease as concentration declines. Of course, this assumes that Canagagigue Creek, Holiday Creek and the Little Ausable River are typical Ontario streams. Correction for

lower temperatures would be based on the general biological relationships (a doubling in rate for each 10°C rise in temperature) until more data are available.

The complex nitrogen transformations which occur in stream water and sediments as demonstrated in the laboratory studies is perhaps another reason for our disappointing field data. Added to further field complexities of ground water, tile drainage and surface inputs, it is not surprising that a budget for such a large flow as that in the East Canagagigue proved so difficult, particularly over short reaches.

It has been demonstrated in this investigation and by others cited throughout this report that streams in Ontario and elsewhere are important sites for removal of some nitrate-N as it is transported to receiving lakes. Agricultural N contribution to lake eutrophication may be lower than previously thought because some of the N is denitrified during transport. The extent of N removal, as shown in this study, is ultimately dependent on the presence of carbon in the stream sediment although the rate appears to be controlled over quite wide ranges of carbon content, by other factors such as temperature and benthic activities. Maintenance of stream bank vegetation is highly recommended to provide a continuous source of carbon to the stream. In streams such as the Canagagigue Creek, however, episodic removal of carbon-rich sediment is common. In such cases some form of hydrologic modification may improve the retention of carbon in the stream bed and, therefore, accentuate the removal of nitrogen.

13.0 Relationship of project results to PLUARG objectives

A major objective of PLUARG was to determine and evaluate the causes, extent and source of pollution from land use activities and to develop an understanding of the relative importance of various land uses in terms of their diffuse pollutant inputs to the Great Lakes. To achieve this objective, there has been an intensive program of monitoring outputs of various land use practices immediately downstream of representative land areas. It is only in this way that pollutant loadings can be directly ascribed to a particular land use. Unfortunately this procedure does not provide precise information on the quantities of pollutants from particular sources which find their way to the lakes. It is recognized that a number of sinks for a variety of pollutants exist in streams, some of these sinks being temporary and some being permanent. Until these are identified and quantified the discharge to the lake of a particular input upstream, i.e. the "delivery ratio" cannot be predicted.

The present work was an attempt to overcome this problem for a particular pollutant, nitrate-nitrogen. It was found that nitrate is, in fact, removed by denitrification during transport in streams at a rate in the order of 0.2 g m^{-2} day^{-1}. It was also shown that nitrate, once in solution in surface water did not enter temporary sinks in the sediment for example by being immobilized in some other form (as NH_4^{-1} or as organic-N) in the sediment. It should therefore be possible to apply the transport factor developed in this work to predict losses of nitrate-N entering upland streams.

References

American Public Health Association. 1971. STandard methods for the examination of water and wastewater. 13th ed. APHA.

Bremner, J.M. 1965 a. Total nitrogen. In Methods of Soil Analysis. C.A. Black (Ed). No. 9 in Agronomy Ser., Amer. Soc. Agronomy, Madison, Wis. pp. 1149-1178.

Bremner, J.M. 1965 b. Inorganic forms of nitrogen. In Methods of Soil Analysis. C.A. Black (Ed). No. 9 in Agronomy Ser., Amer. Soc. Agronomy, Madison, Wis. pp. 1179-1238.

Burford, J.R., and J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biol. Biochem. 7: 389-394.

Fiedler, R. and G. Proksch. 1972. Emission spectrometry for routine analysis of nitrogen-15 in agriculture. Plant and Soil. 36: 371-378.

Fiedler, R. and G. Proksch. 1975. The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: A review. Analytica Chimica Acta 78: 1-62.

Kaushik, N.K. and J.B. Robinson. 1976. Preliminary observations on nitrogen transport during Summer in a small spring-fed Ontario stream. Hydrobiologia. 49: 59-63.

Kaushik, N.K., J.B. Robinson, P. Sain, H.R. Whiteley and W.N. Stammers. 1975. A quantitive study of nitrogen loss from water of a small, spring-fed stream. Proc. 10th Can. Symp. on Water Poll. Res. pp. 110-117.

Liao, C. 1976. (Personal communication). Canada Centre for Inland Waters, Burlington, Ontario

Owens, M., J.H.N. Garland, I.C. Hart, and G. Wood. 1972. Nutrient Budgets in Rivers. Symp. Zool. Soc. Lond. No. 29: 21-40.

Quispel, A. 1947. Measurement of the oxidation-reduction potential of normal and inundated soils. Soil Sci. 63: 265-275.

Rider, B.F., and M.G. Mellon. 1946. Colorimetric determination of nitrites. Anal. Chem. 18: 96-99.

Sain, P., and F.E. Broadbent. 1977. Decomposition of rice straw in soils as affected by some management factors. J. Environ. Qual. 6: 96-100.

Sain, P., J.B. Robinson, W.N. Stammers, N.K. Kaushik and H.R. Whiteley. 1977. A laboratory study on the role of stream sediment in nitrogen loss from water. J. Environ. Qual. 6: 274-278.

Steel, R.G.D. and J.H. Torrie. 1960. Princip;es and Procedures of Statistics. McGraw-Hill, N.Y. 481 pp.

Toms, I.P., M.J. Mindenhall and M.M.I. Harman. 1975. Factors affecting the removal of nitrate by sediments from rivers, lagoons and lakes. Technical Report TR 14. Water Research Centre, Stevenage, Herts., England.

Van Kessel, J.F. 1976. Influence of denitrification in aquatic sediments on nitrogen content of natural waters. Centre for Agriculture Publishing and Documentation, Wageningen.

West, P.W. and T.P. Ramachandran. 1966. Spectrophotometric determination of nitrate using chromotrophic acid. Analytica Chimica Acta. 35: 317-324.

Whistler, F.D., J.C. Lance and R.S. Linebarger. 1974. Redox potentials in soil columns intermittently flooded with sewage water. J. Environ. Qual. 3: 68-74.