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NITRIFICATION IN ONTARIO STREAM SEDIMENTS

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Abstract—Nitrate production by the surface centimetre of stream sediments was investigated in a wide range of sediment types from five areas in southern Ontario. Nitrification rates were estimated in laboratory incubations and ranged from 0 to 180 mg $m^{-2} d^{-1}$. The highest rates were associated with fine textured and organic samples. Nitrate production rate showed a significant correlation with sediment clay content ($r = 0.77$). Many other sediment properties exhibited weaker significant correlations with nitrate production.

Key words—nitrification, nitrate, ammonium, stream sediments, particle size, Ontario, correlation

INTRODUCTION

Studies of nitrogen cycling in stream ecosystems and the associated role of stream sediments have tended to concentrate on nitrate removal processes such as denitrification (e.g. Hill, 1979; Sain *et al.*, 1977). Other processes, linked to nitrate removal, and their control in stream sediments have been studied to a lesser degree (Kaushik *et al.*, 1981).

One such process is the production of nitrate in the aerobic microzone at the sediment–water interface by nitrification of ammonium ions. Studies of nitrification in streams have tended to focus on polluted rivers receiving ammonium from point sources (e.g. Curtis *et al.*, 1975; White *et al.*, 1977). Nitrification also occurs in relatively unpolluted stream systems (Chatarpaul *et al.*, 1979) and lakes (Chen *et al.*, 1972).

Although the influence of sediment properties on nitrogen transformations is often alluded to (Kamp-Nielsen and Moller-Anderson, 1977), few studies have investigated the relationships between nitrification rates and sediment properties. In a comparison of hard and soft water Wisconsin lake sediments Chen *et al.* (1972) found nitrification rates to be suppressed in low pH conditions. Experiments with sediments from the White water river (Kholdebarin and Oertli, 1977) revealed that nitrification rates tended to be greater in the presence of suspended solids, especially when the particle size diameter was greater than or equal to that of the nitrifying organisms. Other factors have been shown to influence nitrification in freshwater sediments such as the presence of tube dwelling worms in the sediment profile (Chatarpaul *et al.*, 1979).

The aims of the present study were to examine nitrate production rates in a wide range of contrasting stream sediments from southern Ontario, Canada, and to investigate the relationships between nitrate production and sediment characteristics.

STUDY AREAS

Areas selected for sediment sample collection are defined in Fig. 1, each containing several sampling sites. The soils, parent material, topography and landuse of the areas have been outlined elsewhere (Wyer and Hill, 1984). Area A was representative of the clay rich till and lacustrine plains south west of Toronto. The poorly drained sand plans to the south of lake Simcoe, area B, provided a source of organic sediments. Area C represented a typical area of coarse textured sand plain. A section of the pre-Cambrian shield north of lake Simcoe, Area D, was selected for soft water sediments. Finally an area of medium texture till landscape was chosen to the east of Toronto, area E.

The streams selected for sediment sampling in each area ranged from small head water streams (<2 m wide) to the middle reaches of moderately large rivers (<30 m wide). This enabled the sampling of a wide range of sediment types throughout the region. In all cases the depth of water was never more than a metre at low flow.

MATERIALS AND METHODS

The research of Curtis *et al.* (1975) revealed that the population of nitrifying bacteria in the sediment and water column of a river peaks in the top centimetre of the sediment profile, in the aerobic layer at the sediment–water boundary. At several sites in each area a portion of the upper few centimetres of the stream bed was carefully extracted using a snow shovel. From this a sample, of approx. 20 cm^2, was taken from the upper centimetre of the profile, placed into

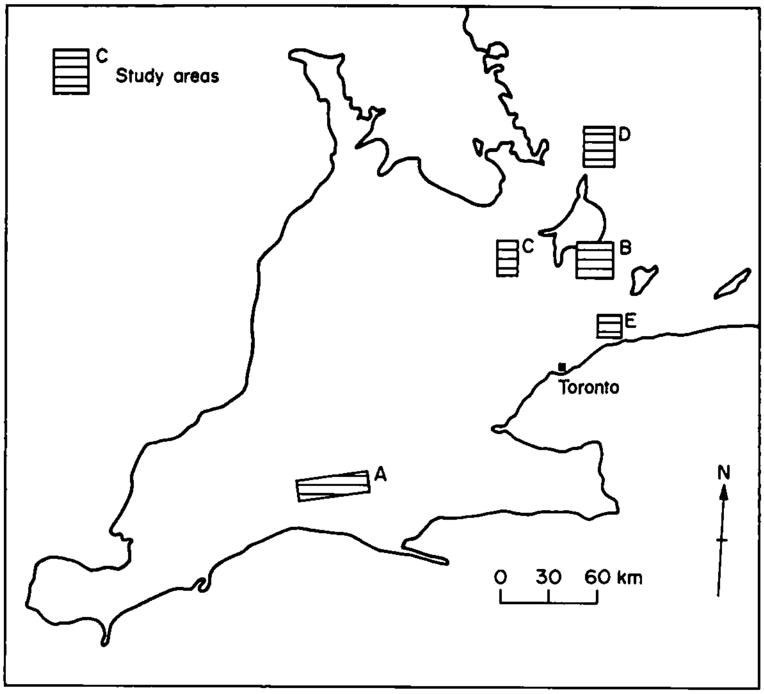

Fig. 1. Location of study areas in southern Ontario.

an acid washed 250 ml Erlenmeyer flask and stoppered. The rest of the surface centimetre layer of the profile was placed into a polyethylene bag for sediment characteristic analysis. The samples were refrigerated in transit and in the laboratory to minimize biological activity prior to analysis. All sampling took place under low flow conditions during one summer season (i.e. under conditions of low disturbance and high temperature, 15–21°C). Each area was sampled in a single day, in the late morning and afternoon. In all 33 sets of samples were taken for analysis. On return to the laboratory 200 ml of distilled water was added to each of the samples in the flasks. Distilled water was used as a diluent in order that all experiments would contain water of similar initial quality. The sealed flasks were then shaken continuously for 6 days at room temperature (21 ± 2°C). Shaking provided turbulent aerobic conditions in the samples, ideal for the process of nitrification. Chen *et al.* (1972) found continuous stirring of lake sediment samples to enhance nitrate production. The incubations were carried out in darkness to minimize biological activity by photosynthetic organisms. A series of duplicate samples containing the nitrification inhibitor N-Serve (2-chloro-6-(trichloromethyl)pyridine) were also run.

Ten millilitre samples were extracted from each flask at intervals of 24, 72 and 144 h from the start of incubation, using sterile glass pipettes. The samples were each treated with a drop or two of 2N KCl as a flocculating agent and centrifuged at 3000 rpm to remove sediment particles from suspension. The supernatant was then analysed for $NO_3 + NO_2$–N using a cadmium reduction method (APHA, 1976) on a Technicon Autoanalyser. NO_2–N was determined by running the samples without the reduction column in line. NH_4–N was assayed using the indophenol blue method (Technicon, 1975).

Sediment pH was determined using a glass electrode on a 1:2 sediment: water mixture. The inorganic nitrogen content of sediment samples was analysed by an extraction using 100 ml 2 N KCl shaken with 10 g of moist sediment. This was followed by filtration and analysis of the resultant solution using the procedures outlined above. Measurement of total nitrogen was carried out using micro-Kjeldhal digestion and analysis of the resultant ammonia content was completed by automated colourimetry (Schuman *et al.*, 1973). Particle size fractions were determined by a combination of wet sieving for sand and the hydrometer method for silt and clay. The Walkley–Black method (Alliston, 1965) was employed to measure the organic carbon content of the sediment samples.

RESULTS AND DISCUSSION

A descriptive summary of the chemical and physical characteristics of the stream sediments sampled in each area is presented in Tables 1 and 2. Although the between area differences could not be tested statistically due to small and variable sample sizes, it appears that the sediments of area A were distinctly rich in silt and clay. Samples from the organic deposits of area B exhibited a comparatively high organic carbon and total nitrogen content. The sediments from area D had a low pH compared to the neutral to basic values typical of other areas. Areas C and E had sediments of high sand content and low ammonium, organic carbon and total nitrogen content.

With the exception of samples from Area D nitrate–N levels showed an increase over time in the shaken sediment water slurries (Figs 2 and 3). The greatest increases were associated with fine and organic sediments (Fig. 2). In duplicate samples incubated in the presence of N-Serve nitrate production was completely inhibited indicating that

Table 1. Mean ($\pm$SD) chemical characteristics of 0–1 cm depth sediments in the study areas

Study area	N	pH	NH_4-N_i ($\mu g^4 g^{-1}$)	NO_3-N_i ($\mu g^3 g^{-1}$)	Total N (mg g^{-1})	Organic carbon (%)
A	4	6.7 ± 0.3	23.8 ± 13.2	0.3 ± 0.2	1.8 ± 0.3	1.9 ± 0.4
B*	5 £4	6.7 ± 0.1	32.5 ± 10.5£[1]	0.3 ± 0.2	5.3 ± 0.4	11.4 ± 6.1
B†	2	7.0 ± 0.0	3.1 ± 2.8	0.1 ± 0.0	0.6 ± 0.2	0.6 ± 0.3
C	8 £7	7.5 ± 0.3	17.8 ± 17.3	0.8 ± 0.7£[1]	0.3 ± 0.2	0.2 ± 0.2
D	6 £5	5.2 ± 1.1	12.0 ± 7.1£[2]	0.2 ± 0.2£[2]	0.5 ± 0.4	1.4 ± 0.6
E	8	7.6 ± 0.2	1.3 ± 1.7	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.3

*Organic sediments.
†Sand plain sediments.
N—No. of samples.
£4—Mean of 4 samples. [1]Extreme values excluded. [2]Extract lost.

Table 2. Mean ($\pm$SD) physical characteristics of 0–1 cm sediments in the study areas

Study area	N	Sand (%)	Silt (%)	Clay (%)
A	4	31.6 ± 16.3	55.4 ± 9.6	13.0 ± 6.9
B*	5	ND	ND	ND
B†	2	94.2 ± 1.6	3.9 ± 1.9	2.0 ± 0.3
C	8	91.7 ± 13.2	6.7 ± 12.2	2.0 ± 0.8
D	6	88.3 ± 5.0	10.0 ± 4.6	1.7 ± 0.3
E	8	90.4 ± 5.1	7.6 ± 4.6	2.1 ± 1.0

*Organic sediments.
†Sand plain sediments.
N—No. of samples. ND—not determined.

the process involved was lithotrophic nitrification. Nitrite–N levels never rose above 0.5 mg l^{-1} and seldom above 0.1 mg l^{-1}.

With the exception of sample B3 which possessed the largest sediment ammonium content of the entire sample set, ammonium–N concentrations were generally low throughout incubation in samples showing the most rapid rises in nitrate–N concentration (Fig. 2). In contrast, samples exhibiting a slower nitrate–N build up, areas C and E (Fig. 3), showed a pattern of increasing ammonium–N levels to the third day of incubation, with a subsequent decline. Samples from area D (Fig. 3) showed a slight increase in ammonium–N levels over time. It was felt that variation in initial ammonium–N concentration from batch to batch of experiments would only be important during the first few hours of incubation. From the onset of incubation microbial processes producing ammonium ions, and diffusion, would be occurring fuelling the nitrification process.

The patterns of change in nitrate–N and ammonium–N over time suggest that rapid con-

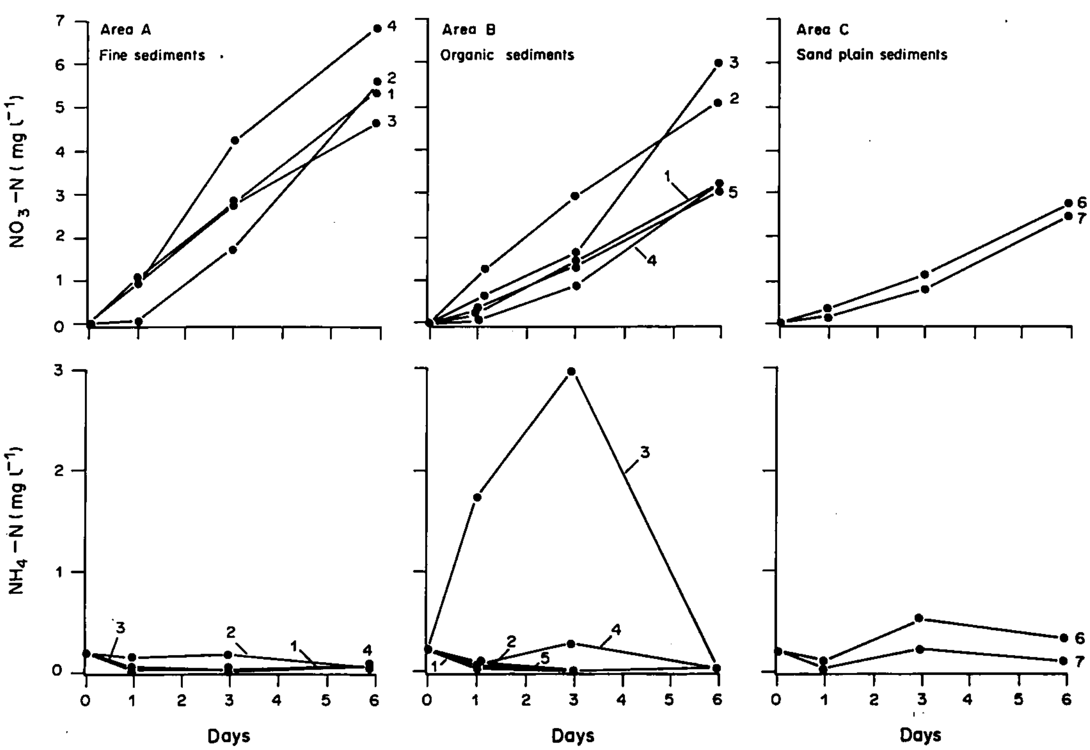

Fig. 2. Nitrate–N and ammonium–N in continuously shaken surficial sediment–water slurries from areas A and B. (1–5 are individual samples.)

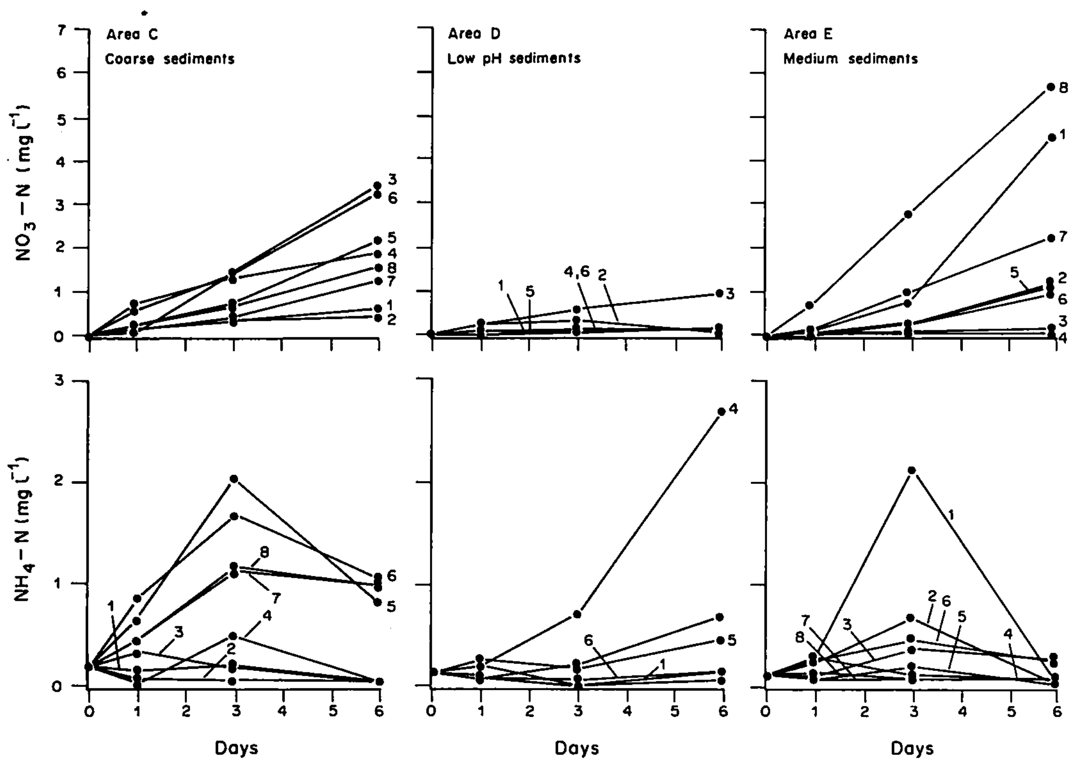

Fig. 3. Nitrate–N and ammonium–N in continuously shaken surficial sediment–water slurries from areas C, D and E. (1–8 are individual samples.)

version of ammonium–N to the nitrate–N form may occur in incubations of fine and organic sediments. These sediments appear to have an abundance of ammonium–N, the energy source for chemautotrophic nitrifiers (Table 1). In incubations of coarser textured and relatively inorganic sediments there is an apparent lag period prior to rapid nitrate production during which ammonium is produced through mineralization of organic material in the sediments. The nitrifier population probably responds to this increase in energy substrate. A similar lag effect is evident from the work of Chen *et al.* (1972) with lacustrine sediments. Because of this apparent batchwise growth of nitrifiers, the overall nitrate production rate may not reflect the initial nitrifier population in these incubations. It is however possible that similar cycles of ammonium production and responding nitrification may occur in the natural river bed. Organic nitrogen inputs for such cycles might include the cyclical growth and decay of stream bed algal communities, Leaf fall, and die back of aquatic vegetation. Thus the overall nitrate production rate may reflect the potential of the sediment for nitrate production. Sediments from the acid shield area showed little evidence of nitrifier activity, as nitrifying bacteria are sensitive to low pH environments (Alexander, 1961).

The nitrate–N levels were used to provide an indication of the nitrification potential of sediments from the contrasting areas by expressing the mean daily accumulation rate on an areal basis (mg m^{-2} d^{-1}). It is difficult to relate the nitrification potentials provided in the laboratory incubations to possible *in situ* nitrification rates in streams. This is due to the artificial nature of the laboratory studies. For example, the shaking of the samples may have provided a more turbulent environment than a stream at low flow, introducing greater amounts of oxygen to the nitrifying organisms. This might lead to an overestimation of environmental nitrification rates. Conversely, the separation of the surface layer from the rest of the sediment profile would have eliminated the supply of ammonium ions from the anaerobic lower profile to the aerobic zone. This might underestimate actual nitrification rates.

The nitrification potentials recorded do, however, provide adequate scope for comparisons between

Table 3. Mean nitrification rates (mg m^{-2} d^{-1}) ($\pm$SD) for 24-, 72-, and 144-h incubations of stream sediments from five contrasting areas of southern Ontario

Study area	*N*	Mean nitrification rate (mg m^{-2} d^{-1}) ($\pm$SD) 24-h	72-h	144-h
A	4	52.5 ± 37.5	74.8 ± 27.0	77.7 ± 12.7
B*	5	57.6 ± 45.2	56.9 ± 36.4	64.8 ± 21.1
B†	2	35.9 ± 12.4	33.9 ± 6.7	42.4 ± 3.0
C	8	16.6 ± 14.6	20.2 ± 14.8	25.6 ± 15.4
D	6	9.0 ± 10.2	10.0 ± 11.6	4.0 ± 5.3
E	8	16.6 ± 22.5	20.2 ± 26.7	28.4 ± 29.5

*Organic sediments.
†Sand plain sediments.
N—No. of samples.

Table 4. Correlation coefficients between daily nitrate–N accumulation, for 24-, 72- and 144-h incubation periods and 0–1 cm depth stream sediment characteristics

Sediment variable	Daily NO_3–N accumulation rate 24-h	72-h	144-h	N
pH (X_1)	0.04	0.04	0.16	33
log organic carbon (X_2)	0.56†	0.58†	0.51†	33
log total nitrogen (X_3)	0.68†	0.60†	0.56†	33
log NH_4–N (X_4)	0.29*	0.42*	0.45*	31
log NO_3–N (X_5)	−0.04	−0.10	−0.12	31
Sand (X_6)	−0.36	−0.64†	−0.70†	28
log silt (X_7)	0.30	0.57†	0.61†	28
log clay (X_8)	0.45†	0.69†	0.77†	28

*Significant at the 0.01 level.
†Significant at the 0.001 level.
N—No. of samples.

sediment types. The mean nitrification rates (Table 3) clearly demonstrate the pattern of high nitrification potential associated with the fine and organic sediments of areas A and B, lower potential with the coarser, inorganic sediments of areas C and E and little nitrification in the low pH sediments of area D. The results also suggest considerable variation in nitrification rates within each area. The range of nitrification rates recorded, 0–128 mg $m^{-2} d^{-1}$, is of a similar order of magnitude to nitrification rates obtained by Chatarpaul *et al.* (1980). These authors recorded values of 29 and 69 mg $^{-2} d^{-1}$ for a pair of Ontario stream sediments using ^{15}N labelled ammonium and optical emission spectrometry.

Relationships between nitrification rates and sediment properties were investigated using Pearson product moment correlation. Many of the independent variables required a logarithmic transformation to improve parametricity (Shaw and Wheeler, 1985). In addition the parametricity of ammonium–N (X_4) and nitrate–N (X_5) was further enhanced by removal of an extreme value in each case (Table 1). Over the full incubation period of 144 h the most significant relationships were found between nitrification rate and particle size fractions (Table 4).

The correlations between nitrification rate and sediment texture are likely to be influenced by multicollinearity between independent variables (Table 5). Correlations between the independent variables suggest that fine textured sediments tend to contain greater amounts of ammonium–N (X_4), organic carbon (X_2) and total N (X_3). This may be due in part to the conditioning of stream bed sediments by local pollution loadings in the areas visited. Isolation of this particular aspect could only be achieved using complex models to predict nitrogen loadings for the streams concerned (Neilsen *et al.*, 1978). This was felt to be beyond the scope of the present study. Correlations between nitrification and sediment texture may thus be partially indirect, in that fine textured sediments tend to possess a larger source of substrate (i.e. organic material for mineralization) for nitrification. High clay and organic matter content may also provide more exchange sites for ammonium ions. This notion is borne out to some degree by the weaker significant positive correlations between nitrate production rate and ammonium–N, organic carbon and total N contents (Table 4). The insignificant correlations between nitrification rates and pH are most likely due to a lack of samples with intermediate pH values between 5.2 and 6.7 (Table 1).

The positive correlation between nitrification and the amount of fine material (silt and clay) in the sediment is consistent with the work of Kholdebarin and Oertli (1977), and suggests a relationship between nitrification and suspended sediment concentration. Suspended sediment concentration is likely to be higher in incubations of fine compared to coarse texture sediments. A fine sediment may also provide a greater surface area for microbial colonization. Nitrifiers could also be particle size specific.

CONCLUSIONS

The results of the present study have demonstrated that, with the exception of low pH sediments, nitrification can occur in a wide variety of stream sediment types, from diverse areas of southern

Table 5. Correlation matrix of stream sediment characteristics, 0–1 cm depth

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
X_1	1	−0.31	−0.10	−0.40	0.21	0.08	−0.24	0.00
X_2		1	0.79†	0.60†	−0.10	−0.64†	0.74†	0.57†
X_3			1	0.46*	−0.06	−0.80†	0.65†	0.77†
X_4				1	−0.21	−0.51*	0.55†	0.53†
X_5					1	0.18	−0.43*	−0.26†
X_6						1	−0.87†	−0.95†
X_7							1	0.78†
X_8								1

*Significant at the 0.01 level.
†Significant at the 0.001 level.

Ontario. Of these, fine and organic sediments possess the greatest potential for nitrification. The dependence of nitrification on sediment properties has been shown. However, it is difficult to isolate the influence of individual variables due to the problem of multicollinearity.

In a parallel study of nitrate removal and sediment properties in the same areas of southern Ontario (Wyer and Hill, 1984) nitrate removal rates were also found to be greatest in fine and organic sediments. This would suggest that fine and organic stream beds are more efficient at cycling nitrogen from an organic state to the atmosphere via the processes of nitrification and denitrification. These stream sediments may thus exert a greater control on water quality than their coarser, less organic and low pH counterparts. Stream reaches with coarse and inorganic acid bed deposits are therefore likely to require more careful management with respect to nitrogen loadings.

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