

## DENITRIFICATION RATES IN RELATION TO STREAM SEDIMENT CHARACTERISTICS

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**Abstract**—Potential rates of nitrate removal were studied in sediments from three Ontario rivers that differed in texture, organic carbon contents and other characteristics. Intact 0–5 cm depth sediment cores from 22 sites on each river were overlain with aerated  $5 \text{ mg l}^{-1} \text{ NO}_3^- \text{ -N}$  solution and incubated in the laboratory at  $21^\circ\text{C}$  for 48 h. Rates of nitrate-N loss from the overlying solutions varied from 37 to  $412 \text{ mg m}^{-2} \text{ day}^{-1}$  for a 24 h incubation period. The acetylene blockage technique was used with nitrate amended sediments to evaluate the relative importance of denitrification and nitrate reduction to ammonium. Denitrification accounted for 80–100% of the nitrate loss in the majority of sediment samples tested. Rates of nitrate loss for the 24 h period exhibited a highly significant positive correlation ( $r = 0.82\text{--}0.89$ ) with the water-soluble carbon content of the sediments in each river. Significant relationships were also observed between nitrate loss and organic carbon, total nitrogen and sediment ammonium. A decline in nitrate loss via denitrification and increased nitrate reduction to ammonium was correlated with the organic carbon and water-soluble carbon content of the stream sediments.

**Key words**—denitrification, nitrate reduction, nitrogen losses, sediments, streams, water quality, water-soluble carbon

### INTRODUCTION

Recent studies of nitrogen mass balances have revealed considerable losses of nitrate during transport in well oxygenated streams draining agricultural and forested landscapes (Hill, 1979, 1981, 1983; Kaushik *et al.*, 1975; Robinson *et al.*, 1979; Swank and Caskey, 1982). Laboratory investigations using either the acetylene blockage technique or labelled  $\text{NO}_3^- \text{ -N}$  have indicated that denitrification in stream sediments is a major factor in stream nitrogen losses (Chatarpaul and Robinson, 1979; Chatarpaul *et al.*, 1980; Hill, 1983; Swank and Caskey, 1982; Van Kessel, 1977a).

Effects of temperature, oxygen and nitrate concentration of the overlying water and the thickness of the sediment layer on denitrification rates in streams have been studied (Sain *et al.*, 1977; Van Kessel, 1977b). The presence of tubificid worms in stream sediments has also been shown to enhance the rate of denitrification (Chatarpaul *et al.*, 1979, 1980). However, relationships between denitrification rates and the chemical and physical properties of stream sediments have received little attention. Swank and Caskey (1982) have indicated that denitrification at 10 sites in a small mountain stream was positively correlated with organic matter and total nitrogen and negatively correlated with sediment nitrate concentrations. Significant but relatively weak correlations between nitrate removal potential of stream sediments and several properties including organic carbon, total nitrogen and sediment ammonium were

observed in laboratory incubations of sediments from many Ontario streams (Wyer and Hill, 1984). However, in this latter study analysis of these relationships was restricted because diverse sediment types, ranging from acidic sands to highly organic muds, were represented by very small sample sizes.

Stream sediment properties may also influence the relative amounts of nitrate which are denitrified or are removed by other nitrate reduction pathways. The disappearance of nitrate in soils and sediments has usually been attributed to denitrification. Nevertheless, several studies have indicated that dissimilatory nitrate reduction to ammonium can be an important process in some carbon-rich, highly anaerobic soils and marine sediments (Caskey and Tiedje, 1979; Koike and Hattori, 1978; Sorensen, 1978; Stanford *et al.*, 1975b).

This paper presents the results of a study designed (1) to examine rates of nitrate loss in relation to the chemical and physical properties of stream sediments and (2) to estimate the relative importance of denitrification and nitrate reduction to ammonium in streams that differ in sediment characteristics.

### STUDY AREAS

Three rivers located near Toronto in Southern Ontario, Canada were selected for study (Fig. 1). The West Humber River drains an area of gently sloping lacustrine clay deposits. The middle reaches of the Nottawasaga River and its major tributaries are located on an extensive level area of outwash sands and gravel, whereas Duffin Creek drains gently to

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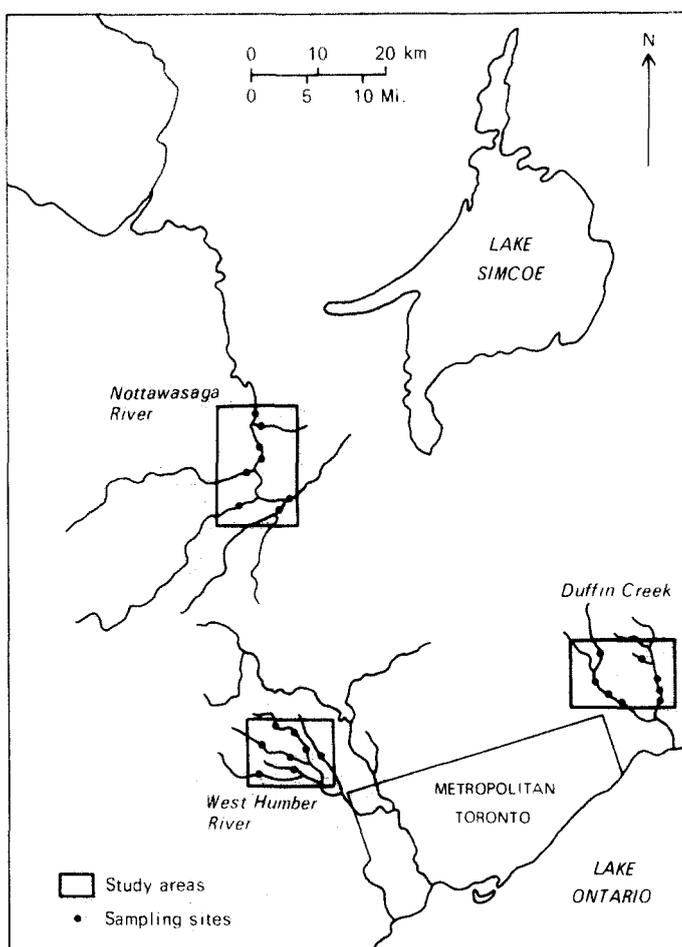


Fig. 1. Location of study areas.

moderately sloping glacial loam till terrain. Land use ranges from mixed farming in the Duffin Creek and West Humber watersheds to heavily fertilized potato cropping on the sand plain adjacent to the Nottawasaga River.

The streams analyzed in the three study areas ranged from small second order tributaries to moderately large sixth order rivers. Stream channel widths varied from 1 to 30 m and water depths were generally <1 m, during low summer flows. Emergent aquatic plants occurred near stream margins in some reaches of the West Humber River but were absent

in the other two study areas. Benthic algae dominated by *Cladophora* sp. and epiphytic diatoms formed mats on gravel riffles in Duffin Creek. Inconspicuous thin algal films occurred on sediments adjacent to stream banks in some reaches of the Nottawasaga and West Humber rivers. Additional details of stream channel characteristics as well as nitrogen mass balance data for Duffin Creek and the Nottawasaga River are given elsewhere (Hill, 1981, 1983).

Sediment characteristics for the stream reaches sampled in each study area are summarized in Table 1. Sediments from the three streams were calcareous

Table 1. Mean ( $\pm$ SD) chemical and physical characteristics of 0–5 cm depth sediments

	West Humber R.	Duffin Creek	Nottawasaga R.
pH	8.0 $\pm$ 0.2	8.2 $\pm$ 0.2	8.4 $\pm$ 0.2
NH <sub>4</sub> -N ( $\mu$ g g <sup>-1</sup> )	8.7 $\pm$ 7.4	5.9 $\pm$ 7.6	0.8 $\pm$ 0.9
NO <sub>3</sub> + NO <sub>2</sub> -N ( $\mu$ g g <sup>-1</sup> )	0.11 $\pm$ 0.23	0.02 $\pm$ 0.04	0.10 $\pm$ 0.11
Total N (%)	0.093 $\pm$ 0.039	0.043 $\pm$ 0.024	0.016 $\pm$ 0.009
Organic-C (%)	1.00 $\pm$ 0.42	0.45 $\pm$ 0.27	0.13 $\pm$ 0.09
Soluble-C ( $\mu$ g g <sup>-1</sup> )	100.0 $\pm$ 44.0	60.2 $\pm$ 27.8	28.6 $\pm$ 11.8
C/N ratio	10.8:1	10.5:1	8.1:1
Gravel + sand (%)	62.1 $\pm$ 14.8	90.7 $\pm$ 4.8	96.0 $\pm$ 2.7
Silt + clay (%)	37.5 $\pm$ 14.9	8.6 $\pm$ 4.8	3.5 $\pm$ 2.7

with a pH of  $>8.0$  but they differed considerably in other characteristics. The Nottawasaga River sediments were coarse-textured with very low levels of organic carbon and ammonium, whereas the West Humber River sediments had a high silt + clay content and organic carbon levels which were about 8 times greater than those in the Nottawasaga River sediments. Sediments collected from Duffin Creek occupied an intermediate position between the other two areas with respect to particle size, organic carbon, sediment ammonium and total nitrogen.

## METHODS

### Sample collection and preparation

Sediment samples were collected during May and June from 8 individual stream reaches in each study area. Sampling was restricted to areas of sediment accumulation in pool reaches and slackwater areas near stream banks. A stainless steel column of 8.5 cm i.d. was used to remove intact cores of 0–5 cm depth from the stream bed at 22 sites in each study area. The cores were gently pushed out of the column into 1000 ml glass beakers with the same internal diameter as the column. Attention was focused on the 0–5 cm depth because several studies have indicated that only the upper 1–5 cm of sediment are involved in denitrification (Sain *et al.*, 1977; Van Kessel, 1977b). Composite samples of the 0–5 cm depth sediment were obtained by combining sediments collected at four points spaced at 90° intervals around each core sampling site. A second set of 0–5 cm sediments was also collected at 12 sites in each study area for estimating the relative importance of denitrification and nitrate reduction to ammonium.

The intact cores were used to evaluate potential nitrate loss rates in stream sediments. The cores were covered with 500 ml of a solution containing  $5 \text{ mg l}^{-1} \text{ NO}_3\text{-N}$  as  $\text{KNO}_3$ . Glass beakers containing only the solution were used as controls. Air was bubbled into the solution close to the water–sediment interface to simulate the turbulence and aeration characteristic of stream conditions. The beakers were incubated in the dark at 21°C for 48 h. Solution samples were removed from each beaker after time intervals of 24 and 48 h. These samples were then analyzed for nitrate + nitrite nitrogen using an automated cadmium reduction method (APHA, 1976). Tests were also made separately for nitrite in the samples. Concentration values were corrected for evaporation losses during the incubation period.

This incubation procedure provides a simple and rapid method for evaluating nitrate removal rates in large numbers of sediment cores. However, research indicates that nitrification in the oxidized surface layer of sediment and denitrification in the deeper anaerobic sediments layers proceed simultaneously (Chatarpaul *et al.*, 1980). Consequently, the disappearance of nitrate from water overlying the sediment cores is underestimated.

The importance of denitrification with respect to nitrate removal by the stream sediments was evaluated using the acetylene blockage technique (Yoshinari and Knowles, 1976). Acetylene inhibits the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  and the resulting accumulation of  $\text{N}_2\text{O}$  can be used as a measure of denitrification activity. Approximately 30 g of fresh 0–5 cm depth sediment was added to 125 ml serum bottles and amended with 15 ml of  $5 \mu\text{g ml}^{-1} \text{ NO}_3\text{-N}$  as  $\text{KNO}_3$  solution. These amounts of sediment and water were sufficient to produce a 1 cm thick layer of water over a similar depth of sediment. The bottles which were capped with septum stoppers remained air-filled and acetylene was added to bring the headspace concentration to 15% (v/v). After mixing the headspace gas by vigorously shaking, the bottles were incubated statically at room temperature for 48 h.

Following incubation gas samples were withdrawn from the headspace and analyzed for  $\text{N}_2\text{O}$  by gas chromatography using a Varian Model 2740 gas chromatograph equipped with a scandium tritide electron capture detector. Nitrous oxide was separated on a Porapak Q column ( $3.3 \text{ m} \times 2.5 \text{ mm}$ ). Temperatures in the injector port, oven and detector were 35, 90 and 240°C respectively. The carrier gas ( $\text{N}_2$ ) flow rate was  $6 \text{ cm}^3 \text{ min}^{-1}$ . Headspace concentrations of  $\text{N}_2\text{O}$  were corrected for  $\text{N}_2\text{O}$  dissolved in the aqueous phase. Total nitrate–N lost was determined by subtracting the amount present at the end of the incubation from the sum of the amounts initially present in the sediment and added in the  $\text{KNO}_3$  solution.

### Analysis of sediment characteristics

The 0–5 cm cores were removed from the beakers after the 48 h incubation period and dried at 50°C for 48 h. Total N in sediment cores was analyzed using a block digester followed by determination of the resulting ammonia on an autoanalyzer (Schumann *et al.*, 1973). Sediment pH was measured with a glass electrode on a 1:2 sediment and water mixture.

Particle size distribution was determined by wet sieving. Organic carbon was estimated by the Walkley–Black method (Allison, 1965). Soluble carbon was determined by water extraction following the procedure of Waring and Gilliam (1983). Organic carbon and water-soluble carbon were also analyzed in sub-samples of the sediments which were used in the  $\text{C}_2\text{H}_2$  incubation experiments.

The initial level of ammonium and nitrate present in the sediment cores was estimated from the composite 0–5 cm depth sediments collected around each core site. Inorganic nitrogen was extracted from the sediment by shaking with 2 M KCl (1/10 w/v) for 1 h. The suspensions were filtered and analyzed for ammonium using an automated indophenol blue method (Technicon, 1975), and for nitrate and nitrite by the automated cadmium reduction method.

## RESULTS AND DISCUSSION

A decline in nitrate concentration occurred in solutions overlying all the sediment cores tested. In some cases nitrate–N declined to  $<0.05 \text{ mg l}^{-1}$  after 48 h. The range of nitrate–N loss based on the surface area of the cores was  $37\text{--}412 \text{ mg m}^{-2} \text{ day}^{-1}$  for the initial 24 h and  $33\text{--}223 \text{ mg m}^{-2} \text{ day}^{-1}$  for the 48 h incubation period. Despite the considerable contrast in sediment properties, rates of nitrate–N loss were similar in the three study areas (Table 2). Cores from sites a few metres apart frequently showed large differences in nitrate loss indicating considerable local variability in nitrate reduction processes within the stream bed. Major differences in nitrate removal also occurred between stream reaches in each study area. For example, nitrate–N losses of greater than  $250 \text{ mg m}^{-2} \text{ day}^{-1}$  in Duffin Creek were associated with cores from pool reaches where sediments were covered by a surface veneer of decomposing algal fragments produced by sloughing of algal mats from upstream riffles. In contrast cores which had nitrate–N losses of  $<100 \text{ mg m}^{-2} \text{ day}^{-1}$  were collected from reaches where benthic algae were absent.

Nitrate loss rates observed in sediments from these three Ontario streams are similar to other studies of stream sediments. Sain *et al.* (1977) recorded losses ranging from 61 to  $165 \text{ mg NO}_3\text{-N m}^{-2} \text{ day}^{-1}$  in a 10 day laboratory incubation with water–sediment

Table 2. Daily nitrate-N loss rates for 0–5 cm depth sediment cores during the initial 24 h incubation period

Site	Mean ± SD	NO <sub>3</sub> -N loss (mg m <sup>-2</sup> day <sup>-1</sup> )				
		0–99	100–199	200–299	300–399	400–499
		Number of sediment cores				
W. Humber R.	172 ± 78	5	11	5	1	—
Duffin Creek	200 ± 104	6	6	5	4	1
Nottawasaga R.	145 ± 93	9	8	3	2	—

profiles. Tracer experiments using <sup>15</sup>N have revealed denitrification rates of 50–90 mg N m<sup>-2</sup> day<sup>-1</sup> (Chatarpaul *et al.*, 1980). In a previous laboratory investigation Wyer and Hill (1984) measured nitrate-N losses of 11–171 mg m<sup>-2</sup> day<sup>-1</sup> for a 24 h incubation period in homogenized 0–5 cm depth sediments. However, disturbance of these samples may have reduced nitrate loss rates. Although it is not possible to make an exact comparison between laboratory experiments and actual rates of nitrate removal in streams, the rates of nitrate loss observed in the laboratory for Duffin Creek and Nottawasaga river sediments are of the same order of magnitude as field losses measured by mass balance techniques in these rivers (Hill, 1981, 1983).

Denitrification measured as the production of N<sub>2</sub>O from acetylene amended sediment samples accounted for >80% of the nitrate loss over a 48 h incubation period in the majority of samples tested (Table 3). The mean (±SD) quantity of N<sub>2</sub>O as a percentage of the nitrate loss was 77 (±11)% in the West Humber river, 80 (±16)% in Duffin Creek and 91 (±6)% in the Nottawasaga river sediments. In 5 of the 24 samples tested from the West Humber and Duffin Creek streams denitrification accounted for only 40–69% of the nitrate consumption.

Previous studies of stream sediments which have involved the addition of <sup>15</sup>N to one or two sediment samples showed that denitrification was responsible for 94–98% of the nitrate loss (Chatarpaul and Robinson, 1979; Van Kessel, 1977a). In contrast, several studies have shown that 20–90% of the nitrate-N applied to lake and marine sediments can be reduced to ammonium and organic nitrogen (Chen *et al.*, 1979; Nishio *et al.*, 1982; Oremland *et al.*, 1984; Sorensen, 1978).

The relatively low proportion of nitrate loss accounted for by denitrification in some sediments from the West Humber and Duffin Creek streams may indicate that dissimilatory nitrate reduction to ammonium is important in these samples. Assimilatory nitrate reduction to ammonium that is incorporated

into microbial biomass can also occur in sediments, however this process is repressed by ammonium (Tiedje *et al.*, 1981). The high ammonium and low nitrate content in the West Humber and Duffin Creek sediments (Table 1) suggests that assimilatory reduction is probably a minor nitrate loss mechanism. Nevertheless, the initial disturbance of the sediments in the serum bottles incorporates nitrate from the solution into the sediment and may also raise the redox potential. These conditions could increase the microbial assimilation of nitrate. If microbial uptake had been enhanced, it is likely that this effect would be most apparent in the sediments from the Nottawasaga River which contained very low levels of ammonium. The importance of denitrification in the Nottawasaga River samples suggests that assimilatory nitrate reduction was not increased in the serum bottle experiments.

It is also possible that the importance of denitrification has been underestimated in some of the stream sediment samples as a result of incomplete inhibition of N<sub>2</sub>O reduction by acetylene. Considerable N<sub>2</sub>O loss in C<sub>2</sub>H<sub>2</sub> amended samples has been observed in soils and sediments particularly at low ambient nitrate concentrations (Knowles, 1979; Oremland *et al.*, 1984; Yeomans and Beauchamp, 1978). In the majority of sediment samples analyzed from the three study areas, <5% of the initial nitrate remained after 48 h of incubation. A comparison of N<sub>2</sub>O concentrations after 24 and 48 h of incubation for 7 sediment samples indicated a decline of 3–13% during the second day of incubation. Three sediments in which N<sub>2</sub>O accounted for <70% of the nitrate loss after 48 h showed a decline of about 5% in N<sub>2</sub>O between the 24 and 48 h samplings.

These data suggest that the production of N<sub>2</sub>O was complete during the first 24 h and that the use of the 48 h incubation period has resulted in an underestimate of denitrification in these sediments. The extent of this underestimation is uncertain, but is probably minor. The losses of N<sub>2</sub>O between the 24 and 48 h samplings are too small in most cases to significantly increase the importance of the denitrification pathway, unless much larger quantities of N<sub>2</sub>O were lost in the initial 24 h of the incubations. This possibility was evaluated by incubating an additional set of 7 sediment samples for 20 h. Approximately 25 and 45% of the nitrate remained in the solution overlying two of these samples after 20 h. Despite these relatively high nitrate concentrations, N<sub>2</sub>O evolution only accounted for 47 and 65% of the

Table 3. Denitrification as a percentage of nitrate-N loss after 48 h of incubation in 12 sediment samples from each study area

Site	N <sub>2</sub> O as a % of nitrate-N loss					
	40–49	50–59	60–69	70–79	80–89	90–100
	Number of samples					
W. Humber R.	1	0	1	4	5	1
Duffin Creek	1	1	1	1	5	3
Nottawasaga R.	0	0	0	0	5	7

Table 4. Correlation coefficients between daily nitrate-N loss during 24 and 48 h incubations and characteristics of 0-5 cm depth stream sediments

Sediment variable	Daily NO <sub>3</sub> -N loss rate					
	West Humber R.		Duffin Creek		Nottawasaga R.	
	24 h	48 h	24 h	48 h	24 h	48 h
pH ( $X_1$ )	-0.65**	-0.55**	-0.62**	-0.55**	-0.31	-0.45*
Organic-C ( $X_2$ )	0.62**	0.58**	0.43*	0.35	0.75**	0.83**
Soluble-C ( $X_3$ )	0.82**	0.73**	0.85**	0.77**	0.89**	0.88**
Log Total N ( $X_4$ )	0.65**	0.59**	0.58**	0.50**	0.68**	0.78**
Log NH <sub>4</sub> -N ( $X_5$ )	0.71**	0.64**	0.76**	0.75**	0.57**	0.67**
Sand + gravel ( $X_6$ )	-0.44*	-0.48*	-0.56**	-0.50**	-0.61**	-0.72**
Log silt + clay ( $X_7$ )	0.49**	0.56**	0.56**	0.50**	0.72**	0.80**

\*Significant at the 0.05 level; \*\*significant at the 0.01 level.

nitrate loss in the two samples. It is unlikely that N<sub>2</sub>O was lost by reduction to N<sub>2</sub> since a considerable quantity of nitrate remained in the solutions. Overall, the experiments with C<sub>2</sub>H<sub>2</sub> amended sediments indicate that denitrification is the major pathway of nitrate removal; other nitrate loss mechanisms, possibly involving dissimilatory nitrate reduction to ammonium may also occur in some sediment samples.

#### Nitrate losses in relation to sediment properties

Correlations between rates of nitrate removal from solutions overlying intact 0-5 cm depth cores and sediment properties are shown in Table 4. Where necessary variables were log transformed to increase the normality and linearity of the data prior to analysis. Sediment nitrate concentration was excluded as a variable because nitrate-N was not detected in more than 50% of the samples from each study area. Daily nitrate loss rates for the 24 h incubation period had a high positive correlation with water-soluble carbon in sediments from the three streams (Fig. 2). Water soluble carbon

accounted for between 67% (West Humber river) and 79% (Nottawasaga river) of the variation in nitrate loss. Nitrate removal from sediments also showed a positive correlation with organic carbon, total nitrogen, sediment ammonium and % silt + clay. Sediment pH had a significant negative relationship with nitrate loss in cores from the West Humber and Duffin Creek streams (Table 4).

The correlations between daily nitrate loss rates for the 48 h incubation and sediment properties were similar to those found for the initial 24 h. As the incubation period lengthened the relationships weakened in the West Humber and Duffin Creek sediments, whereas a small increase in the strength of correlation coefficients was observed for several sediment properties in the Nottawasaga river cores.

The availability of electrons in organic carbon is one of the most important controlling factors in denitrification (Knowles, 1982). In the present study nitrate loss was highly correlated with water-soluble carbon but exhibited a weaker relationship with organic carbon in sediments. Several studies of soils have also revealed that denitrification is more

Table 5. Correlation matrix of stream sediment characteristics (0-5 cm depth) for each study area. Sediment characteristics  $X_1$ - $X_7$  are listed in Table 4

	$X_1$	$X_2$	$X_3$	$X_4$	$X_5$	$X_6$	$X_7$
<i>West Humber R.</i>							
$X_1$	1	-0.85**	-0.84**	-0.88**	-0.77**	0.40*	-0.43*
$X_2$		1	0.90**	0.97**	0.74**	-0.65**	0.68**
$X_3$			1	0.90**	0.87**	-0.60**	0.62**
$X_4$				1	0.77**	-0.64**	0.66**
$X_5$					1	-0.46**	0.48*
$X_6$						1	-0.98**
$X_7$							1
<i>Duffin Creek</i>							
$X_1$	1	-0.77**	-0.76**	-0.78**	-0.70**	0.76**	-0.81**
$X_2$		1	0.72**	0.96**	0.63**	-0.87**	0.85**
$X_3$			1	0.84**	0.88**	-0.71**	0.68**
$X_4$				1	0.76**	-0.89**	0.87**
$X_5$					1	-0.64**	0.61**
$X_6$						1	-0.92**
$X_7$							1
<i>Nottawasaga R.</i>							
$X_1$	1	-0.39**	-0.25	-0.42*	-0.54**	0.34	-0.35*
$X_2$		1	0.81**	0.90**	0.54**	-0.74**	0.79**
$X_3$			1	0.76**	0.61**	-0.63**	0.72**
$X_4$				1	0.58**	-0.75**	0.80**
$X_5$					1	-0.64**	0.68**
$X_6$						1	-0.93**
$X_7$							1

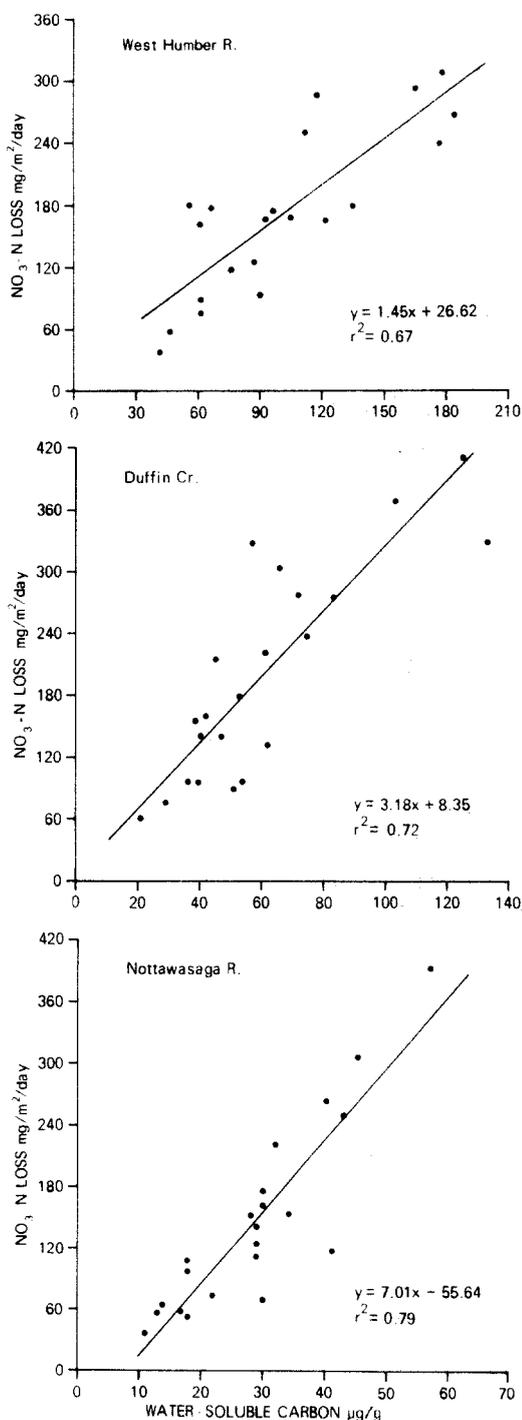


Fig. 2. Relationships between nitrate-N loss during the initial 24 h incubation period and water-soluble carbon content of sediments from the three study areas.

strongly related to a soluble fraction of organic carbon than to total carbon (Burford and Bremner, 1975; Stanford *et al.*, 1975a). A major portion of the organic carbon in soils and sediments is resistant to decomposition. Water-soluble carbon which provides an index of decomposable carbon varied from 0.6 to 5.7% of the organic carbon in sediment cores from the three study areas.

Correlations between nitrate loss and other sediment properties such as total nitrogen, ammonium, pH and texture probably do not indicate causal relationships but instead may be explained by the high degree of interdependence between sediment properties (Table 5). Water-soluble carbon is highly correlated with organic carbon, total nitrogen and sediment ammonium. Silt + clay content is also positively related to nitrogen and carbon whereas sediment pH has a strong negative correlation with water-soluble and organic carbon in the West Humber and Duffin Creek streams. When the effect of water-soluble carbon is removed by partial correlation, relationships between nitrate loss and sediment pH, texture and ammonium are not significant.

A considerable difference in the slope of the regression line for the relationship between nitrate loss and water-soluble carbon is apparent for the three stream systems (Fig. 2). Much higher rates of nitrate loss occur at low levels of water-soluble carbon in the Nottawasaga river sediment cores in comparison to cores from the West Humber river. These data would tend to imply that different amounts of water-soluble carbon can sustain similar rates of nitrate loss in stream sediments. Obviously, other factors are involved. Differences in the depth of nitrate diffusion into the sediment prior to complete removal, and in the vertical profile of water-soluble carbon in the 0–5 cm depth cores could result in similar quantities of readily decomposable carbon being available in sediments from the three streams for use by bacteria involved in nitrate reduction. Experiments with two sediment types containing 20.1 and 11.6 mg of organic matter per gram of wet sediment indicated that a 7 mm layer in the former and a 14 mm layer of sediment in the latter were involved in denitrification (Van Kessel, 1977b).

Differences in the relationship between nitrate loss and water-soluble carbon for sediments from the three study areas could also be influenced by the simultaneous occurrence of nitrification and nitrate reduction during the incubation of the cores. Nitrification activity in the aerobic surface layers of the 0–5 cm depth cores may differ between the three streams. If daily rates of nitrification were higher in sediments from the West Humber in comparison to the Nottawasaga river, this could result in a considerable underestimate of nitrate loss in the former sediments.

Data for the C<sub>2</sub>H<sub>2</sub> amended sediment incubations were used to examine the relation between the organic carbon content of stream sediments and the proportion of nitrate loss which occurred via the denitrification pathway. The proportion of nitrate denitrified after 48 h of incubation showed a significant negative correlation with organic carbon ( $r = -0.70$ ) and water-soluble carbon ( $r = -0.63$ ). A decline in nitrate loss via denitrification and increased NO<sub>3</sub> reduction to ammonium have been

correlated with organic matter levels in soils and marine sediments (Koike and Hattori, 1978; Stanford *et al.*, 1975a, b). Nitrate reduction to ammonium is also enhanced following glucose addition to soils (Buresh and Patrick, 1978; Caskey and Tiedje, 1979).

#### CONCLUSIONS

The results of this study indicate that potential rates of nitrate removal from water overlying intact 0–5 cm depth stream sediment cores were correlated with several chemical and physical characteristics of the sediments. In particular rates of nitrate loss were highly correlated with the water-soluble carbon content of the 0–5 cm sediment depth. Similar magnitudes of nitrate loss were observed despite considerable differences in water-soluble carbon content which ranged from 15–60  $\mu\text{g g}^{-1}$  in the Nottawasaga river to 40–180  $\mu\text{g g}^{-1}$  in the West Humber river sediments. These data suggest that several factors, for example depth of nitrate diffusion into sediment and the relative importance of nitrification–denitrification processes may differ between these streams.

Incubation of sediment using the acetylene blockage technique indicated that denitrification accounted for greater than 80% of the nitrate losses in most sediments. However, nitrate reduction to ammonium may also be important in some sediment samples. The relative importance of denitrification and nitrate reduction to ammonium had a significant relationship with sediment organic carbon. This evaluation of sediment characteristics which affect nitrate reduction in stream sediments shows that the conditions suitable for nitrate removal during transport probably exist in most streams. However, more detailed investigations are needed to clarify relationships between sediment characteristics and nitrogen transformations in streams.

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#### REFERENCES

- Allison L. E. (1965) Organic carbon. In *Methods of Soil Analysis Part 2* (Edited by Black C. A.), pp. 1367–1378. American Society of Agronomists, Madison, Wis.
- APHA (1976) *Standard Methods for the Examination of Water and Wastewater*, 14th edition. American Public Health Association, New York.
- Buresh R. J. and Patrick W. H. (1978) Nitrate reduction to ammonium in anaerobic soil. *Soil Sci. Soc. Am. J.* **42**, 913–918.
- Burford J. R. and Bremner J. M. (1975) Relationships between the denitrification capacity of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biol. Biochem.* **7**, 389–394.
- Caskey W. H. and Tiedje J. M. (1979) Evidence for Clostridia as agents of dissimilatory reduction of nitrate to ammonium in soils. *Soil Sci. Soc. Am. J.* **43**, 931–936.
- Chatarpaul L. and Robinson J. B. (1979) Nitrogen transformations in stream sediments:  $^{15}\text{N}$  studies. In *Methodology for Biomass Determinations and Microbial Activities in Sediments* (Edited by Litchfield C. D. and Seyfried P. L.), ASTM STP 673, pp. 119–127. American Society for Testing and Materials.
- Chatarpaul L., Robinson J. B. and Kaushik N. K. (1979) Role of tubificid worms on nitrogen transformations in stream sediment. *J. Fish Res. Bd Can.* **36**, 673–678.
- Chatarpaul L., Robinson J. B. and Kaushik N. K. (1980) Effects of tubificid worms on denitrification and nitrification in stream sediment. *Can. J. Fish Aquat. Sci.* **37**, 656–663.
- Chen R. L., Keeney D. R. and Sikora L. J. (1979) Effects of hypolimnetic aeration on nitrogen transformation in simulated lake sediment–water systems. *J. envir. Qual.* **8**, 429–433.
- Hill A. R. (1979) Denitrification in the nitrogen budget of a river ecosystem. *Nature* **281**, 291–292.
- Hill A. R. (1981) Nitrate-nitrogen flux and utilization in a stream ecosystem during low summer flows. *Can. Geogr.* **25**, 225–239.
- Hill A. R. (1983) Denitrification: its importance in a river draining an intensively cropped watershed. *Agric. Ecosyst. Envir.* **10**, 47–62.
- Kaushik N. K., Robinson J. B., Sain P., Whiteley H. R. and Stammers W. N. (1975) A quantitative study of nitrogen loss from water of a small spring-fed stream. *Wat. Pollut. Res. Can.* **10**, 110–117.
- Knowles R. (1979) Denitrification, acetylene reduction and methane metabolism in lake sediment exposed to acetylene. *Appl. envir. Microbiol.* **38**, 486–493.
- Knowles R. (1983) Denitrification. *Microbiol. Rev.* **46**, 43–70.
- Koike I. and Hattori A. (1978) Denitrification and ammonia formation in anaerobic coastal sediments. *Appl. envir. Microbiol.* **35**, 278–282.
- Nishio T., Koike I. and Hattori A. (1982) Denitrification, nitrate reduction, and oxygen consumption in coastal and estuarine sediments. *Appl. envir. Microbiol.* **43**, 648–653.
- Oremland R. S., Umberger C., Culbertson C. W. and Smith R. L. (1984) Denitrification in San Francisco Bay intertidal sediments. *Appl. envir. Microbiol.* **47**, 1106–1112.
- Robinson J. B., Whiteley H. R., Stammers W. N., Kaushik N. K. and Sain P. (1979) The fate of nitrate in small streams and its management implications. In *Best Management Practices for Agriculture and Silviculture* (Edited by Lohr R. C. *et al.*), pp. 247–259. Ann Arbor Science, Ann Arbor, Mich.
- Sain P., Robinson J. B., Stammers W. N., Kaushik N. K. and Whiteley H. R. (1977) A laboratory study on the role of stream sediment in nitrogen loss from water. *J. envir. Qual.* **6**, 274–278.
- Schuman G. E., Stanley M. A. and Knudsen D. (1973) Automated total nitrogen analysis of soil and plant samples. *Soil Sci. Soc. Am. Proc.* **37**, 480–481.
- Sorensen J. (1978) Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Appl. envir. Microbiol.* **35**, 301–305.
- Stanford G., Vander Pol R. A. and Dzienia S. (1975a) Denitrification rates in relation to total and extractable soil carbon. *Soil Sci. Soc. Am. Proc.* **39**, 284–289.
- Stanford G., Legg J. O., Dzienia S. and Simpson E. C. (1975b) Denitrification and associated nitrogen transformations in soils. *Soil Sci.* **120**, 147–152.
- Swank W. T. and Caskey W. H. (1982) Nitrate depletion in a second-order mountain stream. *J. envir. Qual.* **11**, 581–584.
- Technicon (1975) *Manual of Methods for Autoanalyzer Analysis*. Technicon, Tarrytown, N.Y.
- Tiedje J. M., Sorensen J. and Chang Y. Y. (1981) Assimilatory and dissimilatory nitrate reduction: perspectives and methodology for simultaneous measurements of several nitrogen cycle processes. In *Terrestrial Nitrogen*

- Cycles, Processes, Ecosystem Strategies and Management Impacts* (Edited by Clark F. E. and Rosswall T.), Ecological Bulletins, Stockholm 33, pp. 331-342.
- Van Kessel J. F. (1977a) The immobilization of nitrogen in a water-sediment system by denitrifying bacteria as a result of nitrate respiration. *Prog. Wat. Technol.* **8**, 155-160.
- Van Kessel J. F. (1977b) Factors affecting the denitrification rate in two water-sediment systems. *Water Res.* **11**, 259-267.
- Waring S. A. and Gilliam J. W. (1983) The effect of acidity on nitrate reduction and denitrification in lower coastal plain soils. *Soil Sci. Am. J.* **47**, 246-250.
- Wyer M. D. and Hill A. R. (1984) Nitrate transformations in southern Ontario stream sediments. *Wat. Resour. Bull.* **20**, 729-737.
- Yeomans J. C. and Beauchamp E. G. (1978) Limited inhibition of nitrous oxide reduction in soil in the presence of acetylene. *Soil Biol. Biochem.* **10**, 517-519.
- Yoshinari T. and Knowles R. (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochem. biophys. Res. Commun.* **69**, 705-710.