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Activities of Benthic Nitrifiers in Streams and Their Role in Oxygen Consumption

A. Bryce Cooper

Water Quality Centre, Ministry of Works and Development, Hamilton, New Zealand

Abstract. The in situ rates of oxygen consumption by benthic nitrifiers were estimated at 11 study sites in 4 streams. Two methods were used: an in situ respiration chamber method and a method involving conversion of nitrifying potential measurements to in situ rates. Estimates of benthic nitrogenous oxygen consumption (BNOC) rate ranged from 0–380 mmol of O_2 $m^{-2} \cdot day^{-1}$, and BNOC contributed between 0–85% of the total benthic oxygen consumption rate. The activity of nitrifiers residing in the sediments was influenced by O_2 availability, temperature, pH, and substrate. Depending upon site, nitrification could approximate either first-order or zero-order kinetics with respect to ammonium concentration. The source of ammonium for benthic nitrifiers could be either totally from within the sediment or totally from the overlying water. Nitrate produced in the sediments could flux to the water above or be lost within the sediment. The sediments could act as a source (positive flux) or sink (negative flux) for both ammonium (-185 $mmol \cdot m^{-2} \cdot day^{-1}$ to $+195$ $mmol \cdot m^{-2} \cdot day^{-1}$) and nitrate ($-135$ $mmol \cdot m^{-2} \cdot day^{-1}$ to $+185$ $mmol \cdot m^{-2} \cdot day^{-1}$).

This study provides evidence to suggest that measurements of downstream mass flow changes in inorganic nitrogen forms may give poor estimates of in situ rates of nitrification in flowing waters.

Introduction

The activities of nitrifying bacteria in converting ammonium to nitrate, via the intermediate nitrite, consume oxygen. In flowing waters that possess high levels of reduced forms of nitrogen relative to levels of reduced forms of carbon (e.g., waters receiving secondary treated effluents), the potential exists for nitrification to be the major process by which dissolved oxygen is consumed. In several streams this potential was apparently realized, with the major dissolved oxygen sink being attributed to benthic nitrification [14, 17]. Effective management of the dissolved oxygen resource of flowing waters, therefore, requires suitable methods to estimate nitrification rates and, for predictive purposes, a knowledge of the factors influencing the magnitude of such rates.

Previous estimates of nitrification rates in streams have been made by following downstream changes in the mass flow of inorganic nitrogen forms, usually nitrate [14, 17, 23]. As nitrification is the only process by which nitrate may be formed, an increase in nitrate mass flow is unequivocal evidence of instream nitrification (assuming other nitrate inputs are accounted for) [20]. However, the *rate* of nitrification estimated from nitrate mass flow data may be seriously awry, when it is considered that nitrate loss mechanisms such as denitrification [18] and assimilation by macrophytes [27] may be occurring in the stream simultaneously with nitrification. Several studies have indicated that measurement of changes in the mass flow of inorganic nitrogen forms is an inadequate method for estimating nitrification rates in flowing waters [5, 23].

In this study, estimates of benthic nitrification rates in 4 New Zealand streams were made using 2 alternative methods. The first method used an in situ benthic respiration chamber, in which nitrification was first permitted and then prevented, using a chemical inhibitor to directly estimate the oxygen consumption due to nitrification. The second method involved converting laboratory measurements of nitrifying potential to estimates of in situ nitrification rate by taking account of the environmental constraints placed upon these potentials. This study also elucidates the factors controlling nitrifier activity in stream sediments, and identifies the sources of ammonium for benthic nitrifiers and the fate of the nitrate which they produce.

Materials and Methods

Study Sites

Studies were carried out on 4 streams in the Waikato and Central Volcanic Plateau regions of the North Island, New Zealand over the period October 1980 to February 1982. At least 5 experimental trips were made to each stream, each trip coinciding with base flow conditions and a recent history (at least 14 days) of stable flows. In a previous study it was shown that stable benthic nitrifying activities could be reestablished within 10–12 days after major storm events [7]. At each location, all experiments were conducted within the same 2.5 m^2 area of stream bed.

The Waiohewa Stream is a small stream (base flow discharge 0.24–0.43 $m^3 \cdot s^{-1}$) that receives inputs of geothermally derived NH_4^+ [6]. No point source effluent inputs occur along its 3 km length or into its headwaters, and levels of biologically oxidizable organics are low [6]. Site WH-A was located 2.5 km from the mouth, where the stream bed consisted of loosely packed, mobile, pumiceous gravel. Site WH-B was located 0.6 km from the mouth, where the stream bed was predominantly coarse sand and was stable under base flow.

The Waiotapu Stream (base flow 2.5–4.4 $m^3 \cdot s^{-1}$) originates as the outlet to eutrophic Lake Ngahewa and empties into the Waikato River 40 km downstream. In its upper reaches, Waiotapu Stream receives inputs from geothermal sources which elevate ammonium levels (20–72 $mmol \cdot m^{-3}$ at WU-A). During spring and summer (October–March) the lower reaches of Waiotapu Stream are depleted in dissolved oxygen as a result of untreated organic waste inputs from a dairy factory. The experimental sites were located 2.5 km upstream from the dairy factory (WU-A), and 3 km and 6 km downstream from the dairy factory (sites WU-B and WU-C respectively). The stream bed at sites WU-A and WU-B consisted of a mixture of pumiceous gravel and coarse sand, whereas at site WU-C the stream bed consisted of sand particles. During stable spring and summer flows the stream bed at site WU-B was partially coated with sewage fungus (dominated by *Sphaerotilus natans*).

The Waitoa River (base flow 0.4–1.4 $m^3 \cdot s^{-1}$) drains an area of intensive dairy farming and, in the study stretch, receives wastes from a dairy factory during spring and summer. Site WA-A was located 1 km upstream of the dairy factory discharge where the stream bed consisted of gravel and coarse sand and was colonized by benthic algae (*Oedogonium* sp.) during summer stable flow conditions. At site WA-B, located 0.05 km downstream from the dairy factory discharge, the stream bed was of coarse sand, and supported prolific benthic growths of sewage fungus (dominated by *Sphaerotilus natans*) during stable flow conditions in spring and summer. Site WA-C was located 10.8 km downstream from the dairy factory discharge where the waters were depleted in dissolved oxygen during summer. The stream bed at this site consisted of compact sand that appeared highly reduced during summer. Site WA-D was located 22 km downstream from the dairy factory discharge in the dissolved oxygen recovery zone. The stream bed at this site was loosely compacted sand.

The Waikare Stream (base flow 0.15–0.4 $m^3 \cdot s^{-1}$) drains an area of peatland used for intensive dairy farming. In the study stretch, the stream receives anaerobic lagoon-treated wastes from a piggery. Sites WE-A and WE-B were located 0.2 km and 1.0 km downstream from the discharge. The stream bed at both sites consisted of coarse, compact sand.

Physical and Chemical Measurements

Measurements of stream temperature, dissolved oxygen, and pH were made using portable meters. Water samples for inorganic nitrogen determinations were pressure filtered (0.45 μm cellulose acetate) in the field and kept on ice until return to the laboratory (<8 hours) at which time they were frozen until analysis. Analyses were performed using automated procedures, with NH_4^+ being determined by a phenol-hypochlorite method [25] and $NO_2^- + NO_3^-$ by a hydrazine reduction method [9].

Sediment Sampling and Analysis

Sediment core samples were obtained (in triplicate) by manually sinking longitudinally-sliced and taped polyvinyl chloride tubing (7.5 cm diameter) into the sediment, placing a rubber bung on top, and carefully withdrawing and inserting another bung at the base of the core before removing from the stream. Overlying water was siphoned off and the core split longitudinally for subsequent depth-interval sediment collection and storage on ice until return to the laboratory. The density and porosity of sediments were determined using weighed subsamples of known volume that were dried to a constant weight at 105°C. Pore water was separated from sediment subsamples by centrifugation (5,000 × *g* for 20 min), and pressure filtered (0.45 μm cellulose acetate). The pH was measured on the pore water sample, which was subsequently stored frozen prior to NH_4^+ analysis as described above. The total NH_4^+ pools (exchangeable + pore water) were determined by extracting sediments for 1 hour with 1 M KCl (1:1, sediment: extract ratio), followed by centrifugation and NH_4^+ analysis of extracts. Exchangeable NH_4^+ was determined by difference between total pool and pore water measurements. The standard deviations of triplicate NH_4^+ analyses (both for pore water and total pool forms) were always less than 8% of the mean values. At the time of sediment collection, the depth of oxygen penetration was taken to be the depth of sediment before black reduced material was observed. Subsamples of the sediment cores were used in assays of nitrifying potential.

Benthic Chamber Experiments

Benthic oxygen uptake rates and inorganic N flux were measured in triplicate at each study site using the in situ experimental chamber described previously [7]. The enclosed streamwater was circulated by a submersible bilge pump, and changes in the concentration of dissolved oxygen (DO) monitored through the in-line incorporation of a precalibrated oxygen probe connected to an oxygen meter. The rate at which water was circulated in the chamber could be varied and was established immediately below the suspension limit of the benthic particulates. Photosynthetic O_2 production was prevented by placing a light-tight box over the chamber. Measurements of O_2 uptake rates were performed with an initial DO concentration in the chamber of greater than 80% saturation and were discontinued when DO concentrations fell below 30% saturation (above 30% saturation rates of O_2 consumption were shown to be independent of DO concentration). At sites where stream DO concentrations were below 80% saturation, the water within the chamber was oxygenated by sparging with compressed air. Repeat experiments on the same area of stream bed were performed by disconnecting the hosing and, with the bilge pump running, expelling O_2-depleted water while drawing in a fresh portion of stream water. After obtaining a total benthic O_2 consumption rate, 30 ml of an aqueous solution of allyl-2-thiorea (ATU) was added to the chamber contents (final concentration 20 $g \cdot m^{-3}$) and, upon establishment of a linear O_2 consumption rate (always within 15 min), a nitrification-inhibited benthic O_2 consumption rate was determined. The slopes of linear regressions obtained for O_2 consumption vs time plots before and after addition of ATU were compared to test for a significant change (at the 95% confidence level) in O_2 consumption rate. The difference in O_2 consumption rate was taken to be that due to benthic nitrogenous oxygen consumption (BNOC) and expressed as mmol O_2 $m^{-2} \cdot day^{-1}$. The error in BNOC rate estimates was highly variable (standard deviations of mean triplicate experiments ranged from 8–65%). Estimates of inorganic N flux were made by comparing the NH_4^+ and $NO_2^- + NO_3^-$ concentrations of samples taken from the chamber at the start and end of the experimental runs in which ATU was absent. The estimates of inorganic N flux based on triplicate experiments possessed standard deviations that were between 8 and 22% of the mean values.

During benthic chamber experimental runs, parallel incubations of stream water in 300 ml bottles were performed to test the likely contribution of suspended microbiota to the observed changes in levels of dissolved oxygen and inorganic nitrogen within the chamber. The results suggest that the aquatic microbiota would have contributed less than 5% to observed changes.

Calculation of Apparent Diffusion Coefficients

Apparent diffusion coefficients for the sediment-water exchange of NH_4^+ were calculated from the estimates of NH_4^+ flux and from the measurements of pore water NH_4^+ concentrations in the uppermost strata of sediment. The following rearranged finite difference form of Fick's First Law was used:

$$D_s = \frac{-F}{\phi \dfrac{\Delta c}{\Delta z}} \tag{1}$$

where D_s is the apparent diffusion coefficient, F is the flux from sediment to water, ϕ is the porosity, Δc is the difference in concentration between the uppermost sediment stratum and the water above, and Δz is the depth from the sediment surface to the midpoint of the uppermost sediment stratum [2, 11].

Assays of Nitrifying Potential

Nitrifying potentials of sediments and stream waters were measured under optimal conditions by incubation of samples (in triplicate) with phosphate buffered (pH 7.2) NH_4^+ (1 $mol \cdot m^{-3}$) solutions in an orbital incubator set at 25°C and 120 rpm [24]. The linear rates of $NO_2^- + NO_3^-$ increase observed for the initial 15 hours of incubation ($r > 0.95$) were taken to be the nitrifying potentials and expressed as mmol N $m^{-3} \cdot day^{-1}$. Assays performed in triplicate demonstrated standard deviations about the means of <7%. The standard conditions of the assay were varied to test the effects of pH, temperature, and NH_4^+ concentration on observed activities. In experiments where the influence of NH_4^+ concentration was being investigated, the NH_4^+ added with the sediment inoculum was taken into account in all calculations.

Results

Estimates of BNOC Rates Using the in situ Chamber

Rates of BNOC for the 11 study sites, as estimated from in situ chamber experiments, varied from 0–380 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$ (Fig. 1). All study sites displayed an apparent seasonal variation in BNOC rates, although the patterns of variation differed. At study sites downstream of organic waste discharges (WU-B, WU-C, WA-B, WA-C, WA-D, WE-A, WE-B), estimated BNOC rates were at a maximum in winter experiments and frequently undetectable in summer. At study sites not influenced by organic waste discharges (WH-A, WH-B, WU-A), maximum BNOC rates were observed in spring and early summer experiments, except for site WA-A where the only experiment of 5 that demonstrated a detectable BNOC rate occurred in winter (May 1981). The contribution that BNOC made to the total benthic oxygen consumption rate ranged from 0–85%, with the maximum contribution at all sites occurring on winter or spring samplings (Table 1). The highest BNOC rates estimated in this study were similar to the oxygen consumption rate measured for some algal and sewage fungus-dominated benthic communities (e.g., Table 2).

Estimates of Nitrifying Potential and Their Conversion to in situ Rates

Nitrifying potentials for stream waters (<3–55 mmol $N \cdot m^{-3} \cdot day^{-1}$) were always less than 2% of the nitrifying potentials obtained for corresponding surface (0–2 cm) sediment samples (360–24,200 mmol $N \cdot m^{-3} \cdot day^{-1}$) (Table 3). Nitrifying potentials can be combined with measurements of environmental parameters likely to restrict nitrifier activity to arrive at a calculated rate for in situ nitrification [15, 16]. To perform such a calculation with my data, the influence of environmental factors on nitrifier activity was determined by manipulation of conditions in the nitrifying potential assay.

The influence of temperature on nitrifier activity obtained in 16 separate experiments (at least 1 sample from each site) over the temperature range encountered in stream surveys (8–25°C), could be explained by a single Arrhenius equation of the form

$$\text{Nitrification rate at } T°C = NP(1.082^{T-25}) \tag{2}$$

where NP is nitrifying potential. Maximal nitrification rate occurred at 25–30°C.

Over the pH range encountered in sedimentary pore waters (pH 5.2–7.2), the dependence of nitrifier activity on pH in all 8 samples tested could be approximated by a single linear regression of the form

$$\text{Nitrification rate at } pH_x = NP[1 - 0.35(7.2 - pH_x)] \tag{3}$$

where NP = nitrifying potential and pH_x = pH of test sample. This linear regression had a correlation coefficient of 0.95 (n = 42).

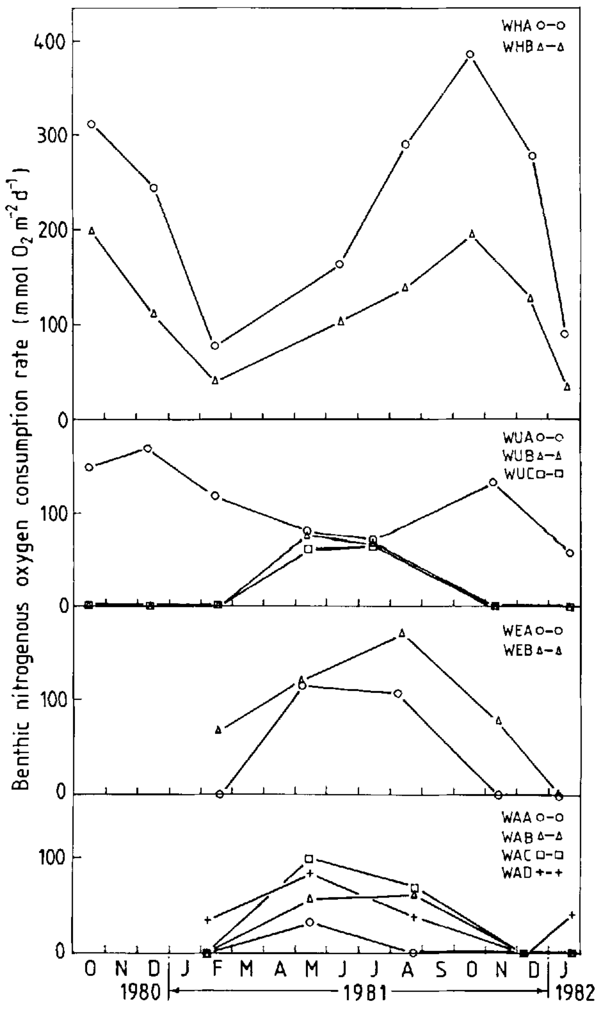

Fig. 1. Seasonal variation in estimates of benthic nitrogenous oxygen consumption rate for the 11 study sites. For site locations and explanation of abbreviations, see Materials and Methods.

Unlike the influences of temperature and pH, the effect of NH_4^+ concentration was strongly site-specific, with the apparent Michaelis-Menten half-saturation constant (K_m) for stream sediments ranging from <4 mmol·m^{-3} to 97 mmol·m^{-3} (Table 4). The dependence of nitrifier activity on NH_4^+ concentration could therefore be described by the Michaelis-Menten equation

$$\text{Nitrification rate at } [NH_4^+]_x = NP\frac{[NH_4^+]_x}{K_{my} + [NH_4^+]_x} \qquad (4)$$

where NP equals nitrifying potential, K_{my} equals half-saturation constant for site y, and $[NH_4^+]_x$ equals total NH_4^+ concentration in test sample. The NH_4^+ pool of oxic sediment strata was measured on 2 occasions at each site and ranged from 6 mmol·m^{-3} to 6,120 mmol·m^{-3} (Table 5). The NH_4^+ pool con-

Table 1. Contribution of benthic nitrogenous oxygen consumption (BNOC) to the total benthic oxygen consumption rate of the 11 study sites

Site	Contribution of BNOC to total benthic O_2 consumption rate (%)	Time of maximal contribution
WH-A	16–85	October
WH-B	10–70	October
WU-A	23–44	July
WU-B	0–22	May
WU-C	0–27	July
WE-A	0–35	August
WE-B	0–49	May
WA-A	0–20	May
WA-B	0–33	August
WA-C	0–33	August
WA-D	0–33	May

Table 2. Estimated rates of oxygen consumption for some different benthic communities sampled during the course of the study

Site	Date	Rate of O_2 consumption (mmol·m^{-2}·day^{-1})	Comments
WA-A	Feb 1981	185	Thin algal film (*Oedogonium*), no nitrification
WA-A	Jan 1982	490	Thick tufts of *Oedogonium*, no nitrification
WA-B	Dec 1981	270	Dark grey-black sewage fungus, no nitrification
WA-B	Jan 1982	1,400	Thick cream-colored carpet of sewage fungus, no nitrification
WH-A	Oct 1981	450	No visible films, 85% of O_2 uptake due to nitrification

sisted of variable proportions of exchangeable NH_4^+ and pore water NH_4^+ (Table 5).

Although nitrifying potential was observed in apparently anoxic sediments, the effective depth of benthic nitrogenous oxygen consumption can be defined by the depth of oxic sediment. At all sites, the depth of oxic sediment was found to vary seasonally, with maxima observed on winter samplings and minima observed on summer samplings.

By combining depth interval measurements of nitrifying potential with measurements of temperature, pore water pH, total NH_4^+ pool, and estimates of

Table 3. Range in nitrifying potentials for surface (0–2 cm) sediment and streamwater samples taken at the 11 study sites over the study period

	Nitrifying potential (mmol $N^{a}\cdot m^{-3}\cdot day^{-1}$)	
Site	Streamwater	Sediment
WH-A	<6–20	3,800–24,200
WH-B	<8–55	3,150–18,250
WU-A	<3–25	3,400–10,850
WU-B	<3–10	1,000–4,640
WU-C	<3–15	800–2,640
WE-A	<6–10	570–6,120
WE-B	<6–10	1,360–4,650
WA-A	<6–10	840–2,140
WA-B	<6–10	360–4,440
WA-C	<6–15	710–5,360
WA-D	<6–18	1,120–4,140

[a] N = nitrogen

Table 4. Apparent half-saturation constants (K_m) and correlation coefficients (r) obtained from Woolf plot transformations ($[S\cdot V^{-1}]$ vs S) of the dependence of nitrifier activity on ammonium concentration for sediments collected from the 11 study sites in May 1981

Site	K_m (mmol $N\cdot m^{-3}$)	r^{a}
WH-A	97	0.98
WH-B	29	0.95
WU-A	64	0.98
WU-B	86	0.97
WU-C	8	0.92
WE-A	<4	–
WE-B	10	0.92
WA-A	61	0.98
WA-B	14	0.93
WA-C	<4	–
WA-D	14	0.98

[a] Linear correlation coefficients obtained using a minimum of 10 data points

the depth of oxic sediment, an estimate of BNOC rate was calculated from the equation:

$$\text{BNOC rate } (\text{mmol } O_2\cdot m^{-2}\cdot \text{day}^{-1}) = 1.9 \sum_{i=1}^{n} NP_iZ_i 1.082^{T-25} \, [1 - 0.35(7.2 - pH_i)]\cdot\frac{[NH_4^+]}{K_m + [NH_4^+]_i} \quad (5)$$

Table 5. Depth profiles of NH_4^+ pools in the oxic sediments of the study sites on two sampling occasions

		Date					
	Sediment stratum	May 1981			January 1982		
Site	(cm)	NH_4^+ tot[a]	NH_4^+ ex[a]	NH_4^+ pw[a]	NH_4^+ tot[a]	NH_4^+ ex[a]	NH_4^+ pw[a]
			$(mmol \cdot m^{-3})$			$(mmol \cdot m^{-3})$	
WH-A	0–2	120	13	107	140	22	118
	2–5	61	6	55	64	9	55
	5–10	6	0	6	–	–	–
	10–15	10	1	9	–	–	–
WH-B	0–2	125	68	57	285	135	150
	2–5	85	43	42	–	–	–
	5–8	124	59	65	–	–	–
WU-A	0–2	40	8	32	42	10	32
	2–5	18	4	14	40	8	32
	5–8	35	7	28	–	–	–
WU-B	0–2	56	5	51	125	11	114
	2–5	61	4	57	–	–	–
	5–8	61	2	59	–	–	–
WU-C	0–2	830	615	215	2,940	1,820	1,120
	2–4	1,020	535	485	–	–	–
WE-A	0–2	705	640	65	3,120	2,510	610
	2–4	980	870	110	–	–	–
WE-B	0–2	510	375	135	1,236	916	320
	2–5	295	224	70	–	–	–
WA-A	0–2	75	5	70	63	4	59
	2–5	122	4	118	–	–	–
WA-B	0–2	565	375	190	–	–	–
	2–4	1,000	625	2,375	–	–	–
WA-C	0–2	845	705	140	6,120	2,895	3,225
	2–4	1,620	1,315	305	–	–	–
WA-D	0–2	225	139	86	1,000	686	314
	2–5	815	685	130	–	–	–

[a] NH_4^+ tot represents NH_4^+ extracted by KCl, NH_4^+ pw represents pore water NH_4^+, and NH_4^+ ex represents exchangeable NH_4^+ obtained as difference between NH_4^+ tot and NH_4^+ pw. All data expressed per cubic meter of total sediment volume

where n = number of depth intervals taken before oxic-anoxic boundary; NP_i = nitrifying potential of the depth interval, i, expressed as mmol $N \cdot m^{-3} \cdot day^{-1}$; Z_i = depth of interval, i, in meters; T = temperature of stream; pH_i = pore water pH of the depth interval i; $[NH_4^+]_i$ = total NH_4^+ concentration in the depth interval i; K_m = half-saturation constant for NH_4^+ oxidation, obtained from Table 4.

The 1.9 multiplication factor converts nitrification rate (as moles of N oxidized per unit time) to an oxygen consumption rate (as moles of O_2 consumed per unit time) [20]. Equation (5) was applied to data from each of 3 cores and the resultant estimates averaged to arrive at a BNOC rate estimate for that site.

Calculated estimates of BNOC rate based upon equation (5) were made on

Table 6. Estimated rates of benthic nitrogenous oxygen consumption (BNOC) calculated from measurements of nitrifying potential and the limitations imposed on this potential in the in situ environment

	BNOC rate (mmol $O_2 \cdot m^{-2} \cdot day^{-1}$)[a]	
Site	May 1981	January 1982
WH-A	6.20 ± 0.31[b]	3.25 ± 0.30
WH-B	3.65 ± 0.22[b]	1.13 ± 0.11
WU-A	2.54 ± 0.35	2.05 ± 0.15
WU-B	3.30 ± 0.32	0.14 ± 0.03
WU-C	2.15 ± 0.21	0.20 ± 0.03
WE-A	3.54 ± 0.21	0.15 ± 0.02
WE-B	3.40 ± 0.41	0.25 ± 0.06
WA-A	1.00 ± 0.11	0.44 ± 0.04
WA-B	1.73 ± 0.30	0.11 ± 0.01
WA-C	2.64 ± 0.28	0.14 ± 0.03
WA-D	2.28 ± 0.23	1.20 ± 0.08

[a] Values presented are means ± 1 SD based on triplicate cores
[b] Sampling date actually June 2, 1981

Table 7. Calculated values of the apparent diffusion coefficient (D_s) for NH_4^+ for the 11 study sites

	D_s ($cm^2 \cdot s^{-1} \times 10^{-3}$)	
Site	May 1981	January 1982
WH-A[a]	11.1	8.4
WH-B[a]	1.3	0.46
WU-A	3.7	3.9
WU-B	6.5	6.8
WU-C	0.11	0.14
WE-A	[b]	0.26
WE-B	[b]	0.37
WA-A	0.27	[b]
WA-B	0.20	0.21
WA-C	[b]	0.02
WA-D	0.32	0.32

[a] Sampling date actually June 2, 1981
[b] D_s not calculated since no flux observed

2 occasions at each site and are presented in Table 6. A non-zero estimate for BNOC rate was obtained on all 22 samplings, with mean estimates possessing coefficients of variation ranging from 5–24% (mean coefficient of variation of 11.7%). In parallel estimates of BNOC rate based upon in situ chamber experiments, there were 8 times when no nitrifier activity could be detected and mean positive estimates on the remaining 14 samplings demonstrated coefficients of variation ranging from 13–65% (mean coefficients of variation of 25%). On all 14 occasions when the 2 methods could be compared, parallel estimates of BNOC rates were not significantly different (*t*-test at 95% level of significance).

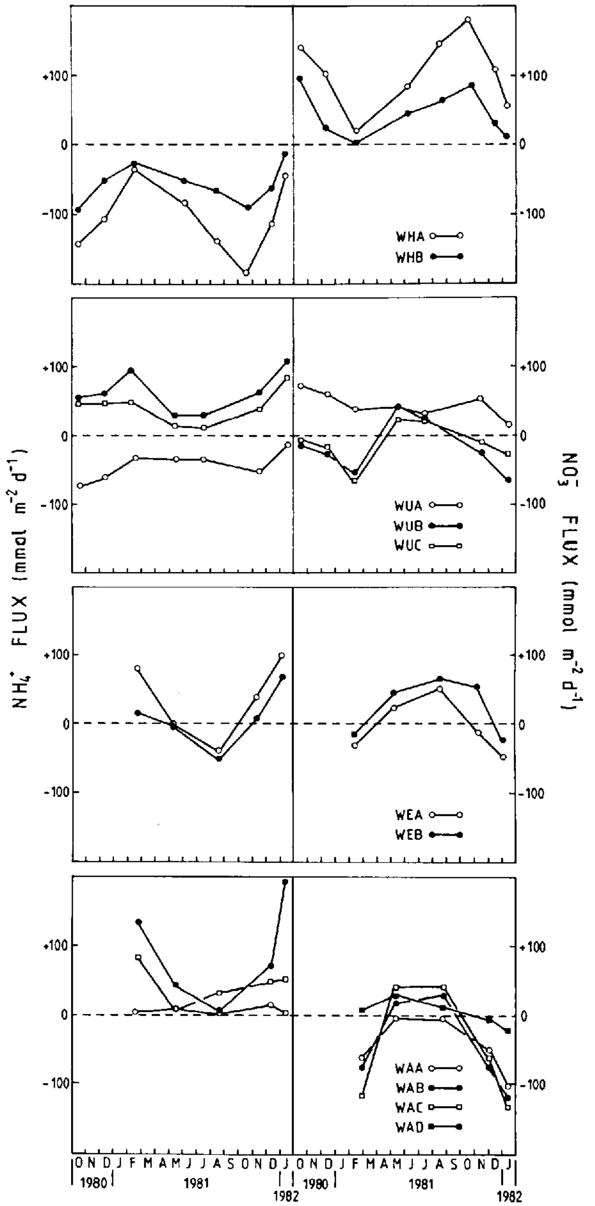

Fig. 2. Seasonal variation in estimates of inorganic nitrogen flux at the 11 study sites. A positive flux is from the sediment to the water.

Estimates of Inorganic Nitrogen Flux

Estimates of inorganic nitrogen flux, based upon changes in the concentration of NH_4^+ and NO_2^- + NO_3^- in the waters of the in situ chamber, are presented in Fig. 2. The stream bed could act as a source (positive flux) or a sink (negative flux) for both inorganic nitrogen species, with NH_4^+ flux ranging from -185 mmol·m^{-2}·day^{-1} to $+195$ mmol·m^{-2}·day^{-1} and NO_3^- flux ranging from -135

$mmol \cdot m^{-2} \cdot day^{-1}$ to $+185$ $mmol \cdot m^{-2} \cdot day^{-1}$. At sites located downstream of organic discharge points there was a flux of NH_4^+ from the sediments, even during winter periods when no effluent was being discharged (except for sites WE-A and WE-B on May and August samplings). At these organically enriched sites, NH_4^+ flux demonstrated seasonal variation with highest positive flux being observed on summer samplings. At sites not influenced by organic inputs, but where streamwaters were enriched with NH_4^+ (WH-A, WH-B, WU-A), the sediments acted as a sink for NH_4^+, presumably because nitrifiers located in the sediment converted the NH_4^+ to NO_3^- thereby maintaining a concentration gradient. The NO_3^- fluxes (but not necessarily their magnitudes) between sites and, temporally, at any one site, were generally opposite to those of NH_4^+ flux. The flux of NO_3^- to sediments was highest at sites that were organically enriched and on summer sampling occasions when the depth of oxic sediment was minimal. The flux of NO_3^- from sediments was highest at sites not influenced by organic inputs (WH-A, WH-B, WU-A) but could occur at organically enriched sites during winter. On some occasions the sediment acted as a source for both NH_4^+ and NO_3^- (e.g., site WU-B on May and July samplings).

Estimates of Apparent Diffusion Coefficients

The estimated values of apparent diffusion coefficient (D_s) for NH_4^+, obtained by use of equation 1, are presented for each site and 2 sampling occasions in Table 7. On 4 occasions, no value for D_s could be calculated, since no measurable NH_4^+ flux occurred. For the 2 sampling occasions, estimates of D_s were quite consistent at any one site (except for site WH-B) but ranged from 0.02×10^{-3} $cm^2 \cdot s^{-1}$ to 11.1×10^{-3} $cm^2 \cdot s^{-1}$ between sites. Highest D_s values were calculated for the loosely packed pumiceous gravels (sites WH-A, WU-A, WU-B), and lowest D_s values were calculated for compact sand sediments (sites WA-C, WU-C).

Discussion

In situ chamber estimates of benthic nitrogenous oxygen consumption (BNOC) rate for the study sites ranged from 0–380 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$ and could account for between 0–85% of the total benthic oxygen consumption rate. By comparison, estimated oxygen consumption rates for benthic algal mats ranged from 185–490 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$, and for benthic sewage fungus communities from 270–1,400 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$ (Table 2). These data demonstrate that nitrification may play an important role in the oxygen-consuming processes of a stream and that, under suitable conditions, the oxygen requirements of benthic nitrifiers can be similar in magnitude to those associated with more visible benthic microbial communities.

Evidence from in situ nitrification studies and assays of nitrifying potentials demonstrated that nitrification in the study streams was principally sediment based. This finding is in agreement with previous studies [8, 21], and indicates that an understanding of factors involved in the spatial and seasonal variation in nitrification rates requires measurement of sediment properties and how they are influenced by sediment-water exchange phenomena. In this study, nitrifying potentials were converted to calculated estimates of in situ benthic nitrification rate by taking into account the limitations imposed on the nitrifiers by certain properties of the sediments (equation 5). This approach has previously been used for marine sediments [15, 16], although these workers did not include the effects of pH and NH_4^+ concentration. In the following paragraphs, the effects of sediment properties in regulating in situ nitrification rates in streams are discussed.

Nitrifying bacteria are strict aerobes and therefore nitrification is restricted to the upper oxygen-containing layer of sediment. At all sites, there apparently was a seasonal variation in the depth of O_2 penetration, with maximum penetration in winter and minimum in summer. The depth to which O_2 penetrates into sediments is controlled by the relative rates of O_2 consumption and O_2 diffusion [12]. During summer samplings, total benthic oxygen consumption rates were at their highest, possibly as a result of high stream temperatures, high microbial biomass, or high organic loadings associated with the seasonal nature of the discharges (or a combination of these factors). A high rate of O_2 consumption presumably leads to the minimum depth of O_2 penetration in summer and, therefore, the minimum volume of sediment that can be a site for nitrification. Despite nitrification being restricted to oxic sediment strata, nitrifying potential was observed in samples collected from anoxic strata. This phenomenon has also been observed in marine sediments [15, 16], and it appears that nitrifiers are capable of surviving periods when oxidative activity is not possible. Nitrifiers collected from anoxic strata began active nitrification immediately upon exposure to oxygen in the nitrifying potential assay (linear rates of $NO_2^- + NO_3^-$ accumulation observed), suggesting that, in the in situ environment, nitrifiers would rapidly take advantage of increases in the depth of oxygen penetration into the sediments.

The influence of temperature on nitrifier activity of sediment samples obeyed the Arrhenius equation, with a maximum activity at 25–30°C and a Q_{10} (proportional increase in activity for a 10° temperature rise) of 2.2. These results are consistent with those previously reported for nitrifier activity in both pure culture and environmental samples [13]. In the streams studied, temperature would not markedly restrict in situ nitrifier activity in summer (stream temperature 20–25°C) but would have been a major limitation in winter (stream temperatures 8–13°C). For example, a temperature of 8°C would limit in situ nitrifier activity to 26% of its potential. Within the in situ sediment environment, however, the overall influence of temperature on areal nitrification rate may be complicated by its parallel influence on other sediment properties. For example, increased temperatures would increase heterotrophic activity within the sediment thereby decreasing the depth of O_2 penetration and thus decreasing the volume of sediment involved in nitrification [15].

The influence of pH in limiting the in situ expression of nitrifying potential would have been of little importance in some sediment strata (pore water pH near optimum of 7.2) but of major importance in other strata. The minimum pore water pH of 5.2 would limit nitrifier activity to 30% of its potential (from equation 5). Although nitrifiers in a particular stratum could be strongly influenced by suboptimal pH, in my study the overall impact of pH on nitrifier activity on an areal basis was unlikely to have been severe, with the likely maximal effect being to limit areal nitrification rates to 78% of potential rates.

Unlike the other regulating factors, the influence of NH_4^+ level on nitrifier activity was strongly site-dependent (Table 4). At some sites (WE-A, WE-B, WU-C, WA-B, WA-C, WA-D), the combination of low half-saturation constants (Table 4) and high NH_4^+ pools (Table 5) led to sediment nitrifier activity being little affected by substrate level (i.e., nitrification rate approximated by zero-order kinetics). At other sites (WH-A, WH-B, WU-A, WU-B, WA-A), the half-saturation constants were similar to or greater than levels of NH_4^+ so that nitrifier activities were limited by substrate levels (i.e., nitrification rate approximated by first-order kinetics). The extent to which areal nitrification rates were restricted by substrate level therefore ranged from no effect (i.e., 100% of potential activity) to a rate that was 24% of the potential. The reason(s) for the wide range in half-saturation constants has not been elucidated. It may be postulated that this range results from variations in the relative proportions of different nitrifying genera, and that these different nitrifiers have markedly different half-saturation constants for NH_4^+ oxidation. Indeed, the existence of multiple genera of nitrifiers has been demonstrated for soils [1] and sediments [6], although reports of comparative studies on their NH_4^+ oxidation kinetics could not be found. An alternative explanation may lie in considering the microenvironment of nitrifiers within the sediments. Nitrifiers residing on sediment surfaces may be placed in a local NH_4^+ environment quite different from that measured for the sediment as a whole.

In this study, sediments at some sites possessed an ability to adsorb a high proportion of the NH_4^+ pool onto their surfaces (NH_4^+ ex, Table 5) and thereby provide a local environment enriched in NH_4^+. Nitrifiers at such surfaces would display NH_4^+ oxidation kinetics in accordance with this local environment and, as a consequence, the apparent half-saturation constants obtained using bulk sediment NH_4^+ concentrations would be lowered. Indeed, those sediments capable of adsorbing a large amount of NH_4^+ onto their surfaces did display the lowest apparent half-saturation constants (cf. Tables 4 and 5). The presence of a locally enriched substrate environment at a surface, and its consequent stimulatory effect on bacterial activities, has been recognized as a mechanism whereby heterotrophic bacterial populations are maintained in substrate-poor environments [10, 22]. It would appear that nitrifiers may utilize the same mechanism.

The importance of ascertaining whether benthic nitrification in streams is approximated by a zero-order or first-order equation becomes apparent when attempts are made to model the process [20]. For water quality managers, the predicted effect of a future increased nitrogenous load on the dissolved oxygen status of a stream could range from no effect (zero-order assumption) to a marked effect (first-order assumption).

By comparing estimates of nitrification rate (Fig. 1) with those of inorganic N flux (Fig. 2), information may be gained on the sources of NH_4^+ for benthic nitrifiers and the fate of NO_3^- produced by their activities. On some occasions, the NH_4^+ flux to the sediment from the stream water could account for all the N used by the benthic nitrifiers (e.g., WH-A and WH-B on all sampling oc-

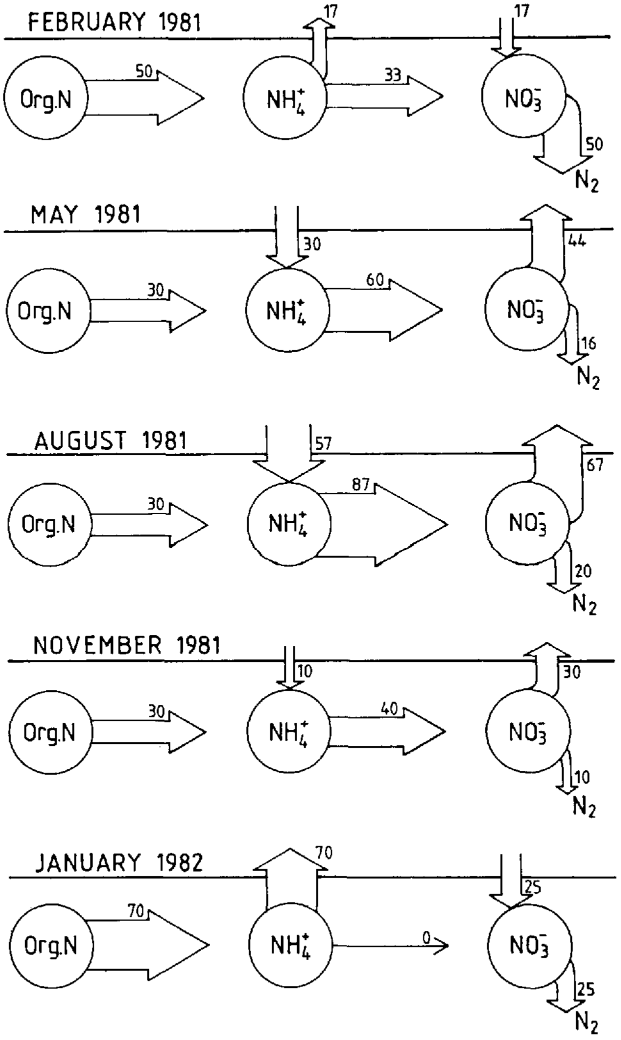

Fig. 3. Model of nitrogen pathways in sediment. Data from site WE-B. All rates in mmol·m^{-2}·day^{-1} of N.

casions). On other occasions, the NH_4^+ flux was from the sediment to the stream water and, therefore, the NH_4^+ for benthic nitrifiers was derived solely from within the sediment by ammonification (e.g., WU-B and WU-C on May 1981 and July 1981 samplings). Between these 2 extremes, there were occasions when a portion of the NH_4^+ required by benthic nitrifiers was supplied by NH_4^+ flux into the sediment and the remainder was supplied by ammonification within the sediment. For example, at site WE-B on the August 1981 sampling, benthic nitrifiers were estimated to be converting 87 mmol·m^{-2}·day^{-1} of NH_4^+ to NO_3^-, of which a measured 57 mmol was being supplied from the stream water and the remaining 30 mmol is assumed to have come from within the sediment.

Of the NO_3^- produced by benthic nitrifiers, a variable proportion (range 0–100%, mean 73%) fluxed to the stream water. The rest of the NO_3^- produced was presumably denitrified within the sediment [16] or taken up by algae residing at the sediment-water interface [11, 19]. Nitrate flux into the sediments could occur, even on occasions when NO_3^- was being produced within the sediments (e.g., WE-A, February 1981). Highest NO_3^- flux to the sediments occurred on occasions when denitrification processes within the sediment would be favored (high organic inputs, minimum depth of O_2 penetration). This suggests that at most sites the NO_3^- loss mechanism of importance was denitrification within the sediment rather than algal assimilation at the interface. An exception was site WA-A, where high NO_3^- loss from the stream water coincided with the presence of a visible algal mat. The sources and sinks of inorganic nitrogen at the study sites can be conveniently displayed in flow diagrams, an example of which is given in Fig. 3.

The results gathered in this study on NH_4^+ and NO_3^- flux and benthic nitrification rates indicate that downstream changes in the mass flow of inorganic nitrogen species are likely to provide poor estimates of in situ rates of nitrification. Due to the simultaneous occurrence of sediment ammonification and denitrification, changes in levels of nitrogen forms in the stream water indicated a lower nitrification rate than that estimated from direct measurements (e.g., Fig. 3). In extreme cases (e.g., Fig. 3, February 1981), changes in levels of nitrogen forms in the stream water were the reverse of those expected for nitrification, and yet nitrification was occurring in the sediment beneath. In the whole stream environment, the inadequacy of a mass flow approach in estimating nitrification rates is likely to be further exacerbated by the ability of aquatic macrophytes to rapidly remove NO_3^- from the stream waters [27].

The estimates of in situ nitrification rate for streams obtained in this study (0–200 $mmol \cdot m^{-2} \cdot day^{-1}$ of N) reached high values, when compared to estimates obtained for marine sediments (0–3 $mmol \cdot m^{-2} \cdot day^{-1}$ of N) [15, 16]. The estimates of NH_4^+ and NO_3^- flux to or from the stream sediments also reached high values (up to 200 $mmol \cdot m^{-2} \cdot day^{-1}$ of N) when compared to estimates for marine sediments (up to 5 $mmol \cdot m^{-2} \cdot day^{-1}$ of N) [3, 4, 11, 19, 26]. Although a number of reasons for such differences in rates can be postulated, a fundamental factor involved is likely to be the difference in exchange phenomena at the sediment-water interface. In essentially quiescent waters (such as those found in some marine environments), the exchange at the interface is principally controlled by molecular diffusion processes, as evidenced by the similarity between estimates of in situ apparent diffusion coefficients and those for molecular diffusion [3, 19]. In systems where the sediment-water interface is disturbed by currents, apparent diffusion coefficients are markedly higher [26]. In this study, crude estimates of the apparent diffusion coefficients for NH_4^+ in the study streams ($0.02–11.1 \times 10^{-3}$ $cm^2 \cdot s^{-1}$) could be several orders of magnitude higher than those found for undisturbed sediments [3, 19]. The wide range in apparent diffusion coefficients suggests that further work in defining the factors controlling diffusivities would lead to a better understanding of the role of benthic microorganisms in modifying the quality of the water that passes above them.

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