Journal of Marine Research, 49, 355–377, 1991

Direct measurement of dissolved inorganic nitrogen exchange and denitrification in individual polychaete (*Nereis virens*) burrows

by Erik Kristensen[1], Mikael Hjorth Jensen[2,3] and Robert C. Aller[4]

ABSTRACT

The burrows of macroinfauna are significant sites of sediment-water nitrogen exchange and associated microbial activity. In this study, the exchange of dissolved inorganic nitrogen (DIN) and nitrogen cycle reaction rates were quantified in individual burrows of the estuarine polychaete *Nereis virens.* Burrow ventilation rate and DIN (NH_4^+, NO_2^-, NO_3^- and N_2O) exchange were determined at 22°C in individual, inhabited burrows with and without the presence of C_2H_2 (an NH_4^+ oxidation, N_2O reduction block). Ventilation cycles were unaffected by C_2H_2, but worm metabolism (O_2 uptake) and excretion of NH_4^+ were enhanced by ~100% and ~50%, respectively. Time-specific DIN exchange patterns were quantitatively modeled by relating burrow water concentration changes, excretion, and ventilation rates. The highest rates were at the start of ventilation periods and decreased or increased (depending on the solute) exponentially to a steady state level. The presence of C_2H_2 increased NH_4^+ release from burrows and changed the NO_2^- flux from a high release (~300 nmol h^{-1}) to an uptake (~ −30 nmol h^{-1}). Nitrate uptake was independent of C_2H_2, presumably because overlying water NO_3^- concentration was high (~100 μM). Indirect estimates of nitrification corresponded to the burrow release of NO_2^- without C_2H_2. Approximately half of the NO_2^- + NO_3^- uptake in burrows was due to denitrification. In microcosms with and without *N. virens* (875 m^{-2}), denitrification was stimulated 3-fold by *N. virens* and the ratio denitrification/nitrification increased from 0.61 to 1.11. The changes in DIN flux and denitrification caused by *N. virens* corresponded well to the rates extrapolated from individual burrows to the appropriate worm density of 875 m^{-2}. At the abundance used, *N. virens* burrows were responsible for 37% and 66% of the total sediment nitrification and denitrification, respectively.

1. Introduction

Infaunal structures, such as burrows formed by bottom-dwelling animals, represent an important mosaic of physico-chemical and biological microenvironments in most coastal sediments. The surface area available for diffusive solute exchange (sediment-water interface area), as well as the area of oxic/anoxic boundaries (redox

1. Institute of Biology, Odense University, DK-5230 Odense M, Denmark.
2. Department of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark.
3. Present address: Institute of Biology, Odense University, DK-5230 Odense M, Denmark.
4. Marine Sciences Research Center, State University of New York, Stony Brook, New York, 11794-5000, U.S.A.

reaction sites) are considerably increased by the presence of ventilated burrows (Hylleberg and Henriksen, 1980; Kristensen, 1984). Oxidized compounds, e.g. O_2 and NO_3^-, are supplied at depth and reduced metabolites, e.g. NH_4^+ and HS^-, are removed by ventilation currents (Kristensen, 1988a; Aller, 1988). This active transport mechanism combined with the usually organic-rich burrow linings (Aller, 1983; Aller *et al.,* 1983), imply that burrow microenvironments generally are sites of elevated microbial activity, heterotrophic as well as chemoautotrophic, compared to ambient sediment or even surface sediment (Aller and Yingst, 1978; Kristensen *et al.,* 1985; Aller and Aller, 1986; Reichardt, 1988). A comprehensive discussion of organic matter decomposition, nutrient cycling and several other aspects of infaunal bioturbation, are given by Aller (1982; 1988), Krantzberg (1985) and Kristensen (1988a).

The estuarine polychaete, *Nereis virens,* which is common in coastal sediments of northern temperate areas (Kristensen, 1988b), has long been recognized as an important bioturbator (e.g. Winston and Anderson, 1971; Kristensen, 1984). *N. virens* ventilates its more or less U-shaped burrows periodically by dorso-ventral body movements. Oxygen transported into burrows by the ventilation current is consumed by the worm itself, by burrow-associated, microbial communities, and by reoxidation of reduced compounds diffusing from the surrounding sediment. The radial penetration depth of O_2 into burrow walls is generally low (e.g. <1 mm) compared to the vertical penetration depth into surface sediment (e.g. 2–5 mm) (Jørgensen and Revsbech, 1985; Reichardt, 1986). The radial diffusion geometry combined with the limited volume of *N. virens* burrows will only allow oxic conditions to persist for very short time, i.e. 5–10 minutes, after the onset of a rest period (Kristensen, 1985; 1989).

Downward transport of NO_3^--rich water by ventilation currents and changing oxic-anoxic conditions over a relatively short time-scale (minutes) control nitrification and NO_3^- reduction (denitrification and NO_3^- ammonification) processes in burrows. Accordingly, the exchange of dissolved inorganic nitrogen (DIN: NH_4^+, NO_2^- and NO_3^-) between *N. virens* burrows and the overlying water, may vary in a highly complex temporal pattern (Kristensen, 1984). Previously, these aspects of bioturbation have mostly been inferred indirectly from microcosms with and without the presence of infauna (Henriksen *et al.,* 1983; Sayama and Kurihara, 1983; Aller and Yingst, 1985; Kristensen, 1985) or by model calculations (e.g. Aller, 1988). However, direct measurements of DIN exchange in individual burrow structures are needed to provide the most accurate assessment of macrofaunal impacts on sediment nitrogen cycling.

The objective of the present study was to quantify the exchange of dissolved inorganic nitrogen and denitrification (by C_2H_2 block) simultaneously in individual *N. virens* burrows. DIN exchange and ventilation rate were measured directly at high temporal resolution during ventilation periods using the V-core set-up of Kristensen (1984). Results from individual burrows were compared to parallel measurements in traditional sediment microcosms with and without the presence of *N. virens.* We had

no intent to determine *in situ* rates, but instead to evaluate the potential influence of *N. virens* on sediment DIN exchange and denitrification. Experiments were therefore conducted at room temperature (22°C) and with NO_3^- enriched overlying water (~100 μM) to enhance process rates and to maintain high pore water NO_3^- concentrations when nitrification was blocked by C_2H_2 exposure (Walter *et al.*, 1979). Such high NO_3^- concentration is, however, within the range previously observed at the collection site and other nearshore Scandinavian regions (Kristensen, 1984; Jørgensen and Sørensen, 1985).

2. Materials and methods

a. Sampling site

The sampling site was near the entrance of the small estuary Norsminde Fjord, Denmark (Stn. 1 of Jørgensen and Sørensen, 1985). The benthic fauna was dominated by the polychaetes *Nereis virens, Nereis diversicolor* and the crustacean, *Corophium volutator.* The most common epifauna were mud snails, *Hydrobia* spp. The density of *N. virens* has previously been found to be 800-900 m^{-2} (Kristensen, 1988b). The annual variation in temperature and overlying water concentration of NO_3^- are 0–20°C and 10–250 μM, respectively (Jørgensen and Sørensen, 1985).

The sediment consisted of low-organic, well-sorted sand. Organic content (loss on ignition at 520°C for 6 h) decreased with depth from 0.7% (upper ½ cm) to a constant level of 0.4% at 4–5 cm depth and below. The organic matter has a molar C:N ratio of around 10 (Andersen and Kristensen, 1988). Porosity decreased from 0.40 in the upper ½ cm to 0.35 at 1–2 cm depth with no further changes below.

b. Sediment collection and handling

Sediment cores and specimens of *Nereis virens* were collected during March. Temperature and salinity were 2°C and 20–23 ‰, respectively. Sediment cores were taken by hand at 0.5 m water depth using 25 by 5.4 (i.d.) cm and 15 by 2.7 (i.d.) cm Plexiglas core liners. The latter cores were assembled immediately after collection two by two using 90° waterpipe fittings (i.d. 3.2 cm) to produce the experimental V-cores (Fig. 1; Kristensen, 1984). Each 'arm' of the V-cores contained similar, undisturbed sediment. The fittings were supplied with rubber O-rings to prevent leakage. All cores were sealed with rubber stoppers and taken to the laboratory. Intact specimens of *N. virens* were collected by digging and gentle sieving.

The impact of *N. virens* on the nitrogen cycle in core microcosms was quantitatively assessed by adding a known number of *N. virens* to defaunated cores. To defaunate cores, O_2 present in the overlying water was stripped off by a stream of N_2 for at least 1 h. The cores were subsequently sealed with rubber stoppers and stored 24 h at 22°C without stirring. The following day, dead and dying infauna were carefully removed without disturbing the sediment. The influence of this defaunation procedure (i.e.

anoxia and the possible presence of free sulfide) on the microbial community was assumed to be insignificant. The overlying water was aerated to resume oxic conditions after defaunation. Although defaunation was efficient, a few infaunal animals were occasionally observed in defaunated cores after the experiments. Data from such cores were omitted.

To each of eight V-cores and ten 5.4 cm cores (designated bulk-cores) were added 1 and 2 individuals of *N. virens* (about 1.5 g wet wt.), respectively. For both core types the added number of worms was equivalent to a density of about 875 m^{-2}. After introduction, the worms rapidly dug into the sediment forming well defined burrows. Visual inspection of cores after the experiments (~ 2 weeks) revealed a mucous-silty inner burrow lining surrounded by a 1–2 mm thick oxidized zone, typical of well established *N. virens* burrows. Although burrows in V-cores were not centered along the entire length of the cores, they were only rarely visible through the transparent core lining. Ten bulk-cores were kept as defaunated controls. All cores were acclimated to the experimental conditions (22°C and 22 ‰) for 8 days in aerated tanks with sea water from the collection locality. The tank water was supplied with NO_3^- on the 7th day to a final concentration of about 100 μM in the water overlying all cores. Every core was thus subjected to similar overlying NO_3^- concentration before initiation of experiments. The long-term (days), transient 'nonsteady state' solute exchange rates, which may occur initially in microcosms used for bioturbation studies (Aller, 1988) are expected to be negligible here due to the long acclimation period.

c. Dissolved inorganic nitrogen (DIN) exchange

V-cores. Four of the 8 V-cores were successful, i.e. the worms made continuous and permanent burrows from one end to the other in the cores. Two days before measurements, V-cores were transferred to two darkened 5-l tanks (similarly enriched in NO_3^-).

The exchange of dissolved inorganic nitrogen, DIN (NH_4^+, NO_2^-, NO_3^- and N_2O), between *N. virens* burrows and overlying water was determined in the presence and absence of C_2H_2 (denoted + and $-C_2H_2$) by monitoring the ventilation currents produced by the worms and relating these to changes in DIN concentrations after water passage through burrows. Acetylene inhibits the reduction of N_2O to N_2 during denitrification, and the rate of N_2O evolution is thus a measure of denitrification (Balderston *et al.,* 1976; Yoshinari and Knowles, 1976). DIN exchange was first determined without C_2H_2 addition in 3 V-cores. The cores were subsequently pre-incubated by purging the 5-l tanks with a mixture of 10% C_2H_2 and 90% air for 20 h and the experiment was repeated. Nitrous oxide was only measured in the presence of C_2H_2. The loss of C_2H_2 to the atmosphere was reduced by sealing the tanks with a gastight lid that only allowed passage of the necessary wires and tubes. A

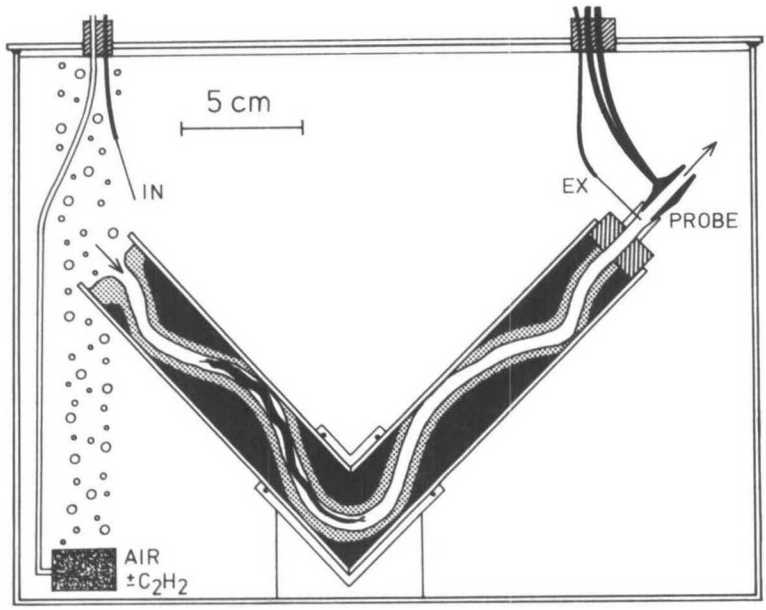

Figure 1. The experimental V-core, showing a *Nereis virens* inhabited burrow in undisturbed sediment. Arrows indicate the direction of the ventilation current; IN and EX are incurrent and excurrent sampling ports, respectively. Cables from the flowprobe are connected to an electromagnetic flowmeter (for further details see text).

slow stream of air ($-C_2H_2$) or C_2H_2/air mixture ($+C_2H_2$), respectively, was maintained throughout the experiment.

The ventilation rate was measured with an electromagnetic flowmeter (Gould SP2202 Blood Flowmeter) equipped with a 0.5 cm i.d. flowprobe and recorded by a Phillips PM 8222 Dual-pen Recorder. A 2-cm polyethylene tube connected the worm burrow with the flowprobe through a single-hole rubber stopper (Fig. 1). The stopper was carefully inserted into one arm of the V-core until it approached the sediment surface such that the tube was continuous with and acted as an extension of the burrow (Kristensen, 1984). Water samples for incurrent and excurrent DIN analysis were taken by syringe from the overlying water close to the (worm)head end and from the connecting tube before the flowprobe at the tail end of the V-core, respectively (Fig. 1). Sampling just before the flowprobe when the ventilation record showed positive flow, ensured that only excurrent water was extracted. The first excurrent sample was always taken within a few minutes after the onset of a ventilation period (a total of 3–7 samples per ventilation period). Each V-core incubation lasted for about 3 h during which 2 or 3 ventilation periods were monitored successfully.

Excurrent water samples of 1-ml from the untreated cores ($-C_2H_2$) were transferred to scintillation vials and frozen immediately (−20°C), while 1-ml samples from

C_2H_2 treated cores ($+C_2H_2$) were injected into pre-evacuated, 3-ml 'Venoject' tubes (Terumo Europe N.V., Leuven, Belgium). The remaining 2-ml vacuum was equalized with pure N_2 and the samples were immediately frozen (−20°C). Similar samples were taken from the overlying water 4–5 times during each V-core incubation for determination of incurrent DIN concentrations. Incurrent DIN levels were constant within each incubation period although they differed slightly between the various incubations. Incurrent concentration ranges for all incubations + and $-C_2H_2$ were: 45–60, 5–5.5, 95–108 and 0.1 μM for NH_4^+, NO_2^-, NO_3^- and N_2O-N, respectively. Elevated NH_4^+ concentrations (2–3 times the average level at the sampling site) were due to NH_4^+ release from the sediment cores during the initial acclimation period.

Bulk-cores. DIN flux and dentrification were measured in twelve 5.4 cm cores (bulk-cores), 6 with *N. virens* and 6 defaunated controls; 3 of each were incubated with C_2H_2 and the remaining without. Pre-incubations of $+C_2H_2$ cores were carried out in tanks as described for the V-cores, i.e. 20 h in a 1:9 C_2H_2/air mixture. Initial DIN concentrations in the water overlying the bulk-cores were similar to those in the (incurrent) water overlying the V-cores.

Immediately after the initial water samples of 1 ml were taken from the tanks, all cores were closed with rubber stoppers and incubated with continuous stirring for about 2 h in the dark. The height of the water phase in the cores was 4–5 cm (equal to 90–115 ml of water). Oxygen was expected not to decrease below 70% of saturation and DIN exchange was assumed constant during the 2-h incubation period. One-ml samples taken after 2 h represented the final DIN concentration in the overlying water. All samples + and $-C_2H_2$ were treated as described for the V-core experiment.

After the final water sampling, the C_2H_2-exposed sediment was quickly cut into 0.5-cm (upper cm) or 1-cm (1–9 cm) sections. Accumulated N_2O was extracted by the headspace technique of Andersen *et al.* (1984). Briefly, extruded sediment sections were quickly transferred to pre-weighed 60-ml beakers containing 5 or 10 ml 2 M KCl. The beakers were immediately closed with rubber stoppers, reweighed and shaken vigorously for 2 min. Three-ml samples of the headspace gas were transferred to pre-evacuated 'Venoject' tubes for analysis of N_2O. The initial ('time zero') N_2O content in C_2H_2-inhibited sediment was determined similarly in 4 separate bulk-cores (2 with and 2 without *N. virens*), which also had been subjected to 10% C_2H_2 for 20 h.

The present version of the C_2H_2 block technique differs from previous *in situ* applications; i.e. needle-injection of small portions of the inhibitor into closed sediment cores followed by a short incubation immediately afterwards (e.g., Sørensen, 1978; Andersen *et al.*, 1984; Jensen *et al.*, 1988; Jørgensen and Sørensen, 1988). Here the cores were left open in a C_2H_2-sparged reservoir for a period of time sufficient to ensure an even distribution of C_2H_2 in the denitrifying sediment volume

prior to incubation. This modification was used in order to avoid any damage to the worms by a needle. The NO_3^- enrichment, that assured relatively high pore water NO_3^- concentrations in both cores + and $-C_2H_2$, allowed the long pre-incubation.

d. NH_4^+ excretion by N. virens

The contribution of excretion by *N. virens* to the measured NH_4^+ flux was determined both with and without C_2H_2 in a separate experiment. After collection, worms were acclimated to the experimental temperature (22–23°C) in natural sediment for 5 days. About 24 hours before use they were removed from the sediment and each worm was allowed to inhabit a clean polyethylene tube of appropriate size. Batches of 2–3 individuals (each about 0.5–1 g wet wt.) were incubated in sealed and stirred 500-ml glass bottles containing 18 ‰ sea water. Six batches were purged with a gas mixture of 10% C_2H_2:90% air for 20 h before incubation, whereas another 6 batches remained untreated in fully oxic water. Initial and final samples for O_2 and NH_4^+ were taken from the bottles before and after an incubation period of 4–6 h in the dark. The samples for NH_4^+ analysis were frozen immediately. Oxygen was analyzed by the standard Winkler technique.

e. DIN analysis

The 'Venoject' tubes containing 1-ml water samples were quickly thawed in a water bath at room temperature (22°C) and vortexed for 2 min for equilibration of dissolved N_2O with the headspace gas. These samples and gas samples from sediment extractions were analyzed for N_2O content by injection of 0.2 to 0.3 ml subsamples onto a Packard model 427 gas chromatograph equipped with a ^{63}Ni Electron Capture Detector held at 320°C. A backflush system prevented C_2H_2 contamination of the detector and allowed for a short analysis time (3 min). Gases were separated on a 80/100 mesh Porapak Q column (2-m long and 3.2-mm wide) operated at 80°C. Pure N_2 was used as the carrier gas at a flow rate of 15 ml min^{-1}. 'Venoject' tubes containing water samples were quickly refrozen after N_2O analysis. The content of N_2O in sediment and water samples was obtained from the measured N_2O concentration in the headspace using solubility coefficients for N_2O in KCl solution or sea water (Markham and Kobe, 1941; Weiss and Price, 1980).

All water samples were later analyzed using the standard autoanalyzer methods of Solorzano (1969) for NH_4^+ and Armstrong *et al.* (1967) for NO_2^- and NO_3^-.

f. Rate calculations

The rates of NH_4^+, NO_2^- and NO_3^- exchange in bulk-cores were determined from the concentration changes in the water phase during incubation. Denitrification rates in C_2H_2 treated bulk-cores were determined as the total accumulation of N_2O in sediment and overlying water corrected for the initial ('time-zero') content. Esti-

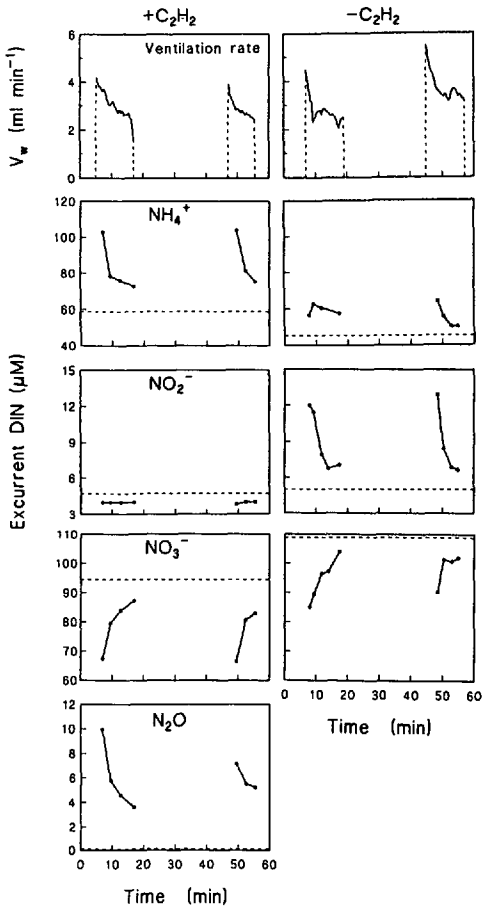

Figure 2. Examples of time-specific ventilation pattern and excurrent DIN (NH_4^+, NO_2^-, NO_3^-, N_2O-N) concentrations during 1 hour in a *Nereis virens* inhabited V-core in the presence (left) and absence (right) of C_2H_2. Records of two ventilation periods are shown. The broken horizontal lines indicate the incurrent DIN concentrations.

mates of DIN flux and denitrification in individual burrows required more complex model calculations (see below).

3. Results

a. Ventilation and excurrent DIN patterns in V-cores

A representative example of ventilation and excurrent DIN concentration patterns from the V-core incubations is shown in Figure 2. Ventilation was intermittent, interrupted by periods of rest in a more or less rhythmic fashion. The presence of C_2H_2 did not affect the natural ventilation pattern (Table 1). The average duration of ventilation periods (t^*) for all examined *N. virens* in the V-core incubations were

Table 1. Ventilation pattern and burrow dimensions in the experimental V-cores (both data for cores with and without C_2H_2 is presented). D_v is the fractional time a burrow is ventilated; t^* is duration of ventilation periods; V_w is ventilation rate during active periods; r_B is radius of burrows; V_B is water volume of burrows excluding the volume of the worms; A_B is surface area of the burrow wall. All values are mean (S.D.) of 3 V-cores.

Treatment	D_v	t^* (min)	V_w (ml min^{-1})	r_B (cm)	V_B (cm^3)	A_B (cm^2)
$-C_2H_2$	0.26	14.0	3.0	0.30	4.8	42.2
	(0.05)	(4.3)	(0.4)	(0.03)	(0.9)	(5.4)
$+C_2H_2$	0.28	14.5	3.1	0.29	4.7	41.5
	(0.07)	(2.6)	(0.1)	(0.03)	(0.9)	(5.4)

14–15 min and burrows were ventilated 26–28% of the time ($100 \cdot D_v$) at the actual temperature. Average ventilation rate was about 3 ml min^{-1}. Although there was no noticeable affect of C_2H_2 on ventilation behavior, the metabolic rate of the worms measured as O_2 uptake almost doubled from 3.2 to 6.2 μmol $g^{-1}h^{-1}$ when C_2H_2 was added. This was associated with a 50% stimulation of the average NH_4^+ excretion rate from 660 ± 180 to 960 ± 300 nmol $g^{-1}h^{-1}$.

Water passage through burrows in V-cores affected the concentration of all DIN species both with and without C_2H_2 in a general pattern (Fig. 2). The difference between excurrent and incurrent DIN concentrations during ventilation periods decreased exponentially with time and approached a steady state level. Ammonium release from burrows was generally higher with C_2H_2 than without. A part of this was due to a stimulation of NH_4^+ excretion by *N. virens*. Nitrite was taken up slightly in the presence of C_2H_2, but was otherwise rapidly released. Despite this dramatic change, a net consumption of total oxidized nitrogen ($NO_2^- + NO_3^-$) always occurred in burrows both with and without C_2H_2. Nitrous oxide production in burrows was significant in the presence of C_2H_2; for example, as shown in Figure 2, excurrent concentrations ranged from 3 to 10 μM N_2O-N compared to an incurrent N_2O-N of only 0.1 μM.

b. DIN exchange in V-cores

Exchange-model. The exchange of DIN in an infaunal burrow is controlled by both reaction and transport processes in the surrounding sediment (Aller, 1980). The build-up or consumption of DIN species that occur during ventilatory rest periods (Fig. 3) is caused by microbial processes and diffusion in the more or less anoxic burrow wall, and in the case of NH_4^+ also by excretion of the inhabitant worm. Dilution of burrow water with overlying water is responsible for a rapid exponential change (increase or decrease) in solute concentration during the initial part of an active ventilation period. The subsequent steady state concentration level that is approached in the water exiting a burrow late in ventilation periods represents the

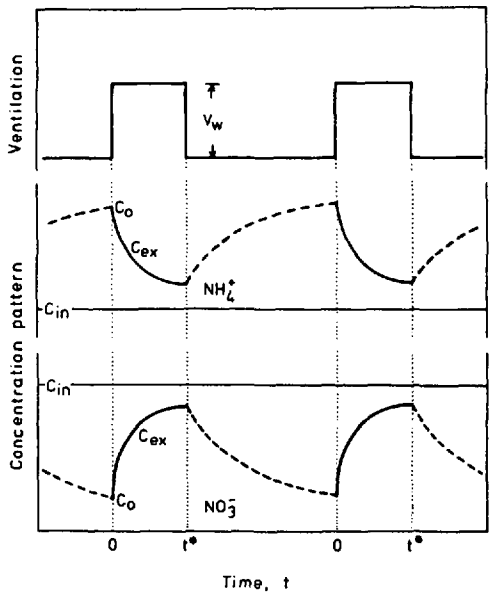

Figure 3. Idealized ventilation pattern (square wave), V_w, and time-specific excurrent, C_{ex}, concentration change of NH_4^+ and NO_3^- as proposed by the model in Eq. 1–10. The broken lines indicate the expected concentration change in burrow water during rest periods. C_o is the concentration in excurrent water at the start of ventilation periods ($t = 0$) or the concentration in burrow water at the end of rest periods. C_{in} is the incurrent DIN concentration and t^* indicate the end of ventilation periods.

balance between diffusion controlled reaction rates in the oxic burrow wall (+worm excretion) and the advective transport mediated by the ventilating worm.

The time rate of solute concentration change in water exiting a nereid burrow during active ventilation (nmol ml^{-1} min^{-1}) is given by the following transient-state form of the burrow water models used by Aller *et al.* (1983):

$$dC_{ex}/dt = C_{in}V_w/V_B - C_{ex}V_w/V_B + J^wA_B/V_B + R_{nv} \quad (1)$$

where:

C_{ex} = solute concentration in water exiting burrow (nmol ml^{-1})
C_{in} = solute concentration in water entering burrow (nmol ml^{-1})
V_w = ventilation rate during active periods (ml min^{-1})
V_B = volume of water in burrow (burrow volume minus volume of the inhabitant worm) (cm^3)
A_B = burrow wall surface area (cm^2)
J^w = flux of solute from burrow wall (nmol cm^{-2} min^{-1})
R_{nv} = excretion of solute by *Nereis virens* (nmol (cm^3 burrow water)$^{-1}$ min^{-1})

The basic assumptions are that the burrow water is well mixed at any given time; that

the ventilation rate (V_w) is approximately constant during an active ventilation period (i.e. a square wave, see Fig. 3); and that the burrow wall solute flux (J^w) and worm excretion (R_{nv}) is constant for the bulk of the ventilation period.

If $C_{ex} = C_o$ when $t = 0$ (start of ventilation period), then:

$$C_{ex}(t) = C_{in} + J^w A_B/V_w + R_{nv}V_B/V_W + (C_o - C_{in} - J^w A_B/V_w - R_{nv}V_B/V_w)e^{-tV_w/V_B} \quad (2)$$

Defining: $\tau = V_B/V_w$ (residence time of water in burrows) and $\Delta C(t) = C_{ex}(t) - C_{in}$, then:

$$\Delta C(t) = J^w 2\tau/r_B + \tau R_{nv} + (C_o - C_{in} - J^w 2\tau/r_B - \tau R_{nv})e^{-t/\tau} \quad (3)$$

where r_B = burrow radius (cm).

The solute concentration in burrows (C_o) at the end of rest periods or at the start of ventilation periods (t = 0) can be derived from Eq. 2. Note that as $t/\tau \rightarrow$ large (late in ventilation period) then the steady state excurrent solute concentration is:

$$C_{ex}(t) = C_{ex}^{ss} = C_{in} + J^w 2\tau/r_B + \tau R_{nv} = \text{const.} \quad (4)$$

Let the solute concentration in the final sample (t_f) during ventilation periods be: $C_{ex}^{ss} = C_{ex}(t_f)$, and let the solute concentration in the initial sample during ventilation periods (e.g. $t \approx 2$ min) be: $C_{ex}(t_i)$, then:

$$C_{ex}(t_i) = C_{ex}(t_f) + (C_o - C_{ex}(t_f))e^{-t_i/\tau} \quad (5)$$

and

$$C_o \approx (C_{ex}(t_i) - C_{ex}(t_f)(1 - e^{-t_i/\tau}))e^{t_i/\tau} = C_{ex}(t_i)e^{t_i/\tau} - C_{ex}(t_f)(e^{t_i/\tau} - 1). \quad (6)$$

The value of C_o affects the total (integrated) flux of solutes out of and into burrows (see later), but does not influence estimates of the steady state flux across the burrow wall during ventilation periods (J^w). Because τ is small (1–2 min) relative to t^* (14–15 min) then the excurrent solute concentration (C_{ex}) reaches a steady state value to a reasonable approximation when t is close to t^* (assuming that J^w and R_{nv} are constant). J^w during ventilation can therefore be obtained from the mass balance in Eq. (4) according to:

$$J^w = (C_{ex}^{ss} - C_{in})r_B/2\tau - r_B R_{nv}/2. \quad (7)$$

The water volume (V_B) and radius (r_B) of the present burrows (Table 1) are estimated from the empirical relationship between body weight of *N. virens* and burrow dimensions as reported by Kristensen (1984), i.e. $V_B = 3.76W^{0.709}$ (W is wet weight of worms) where burrow length is between 15 and 20 cm. Ammonium excretion by *N. virens* (R_{nv}) was measured to be around 2.5 and 3.5 nmol cm^{-3} min^{-1} for worms without and with 20 h pre-exposure to 10% C_2H_2, respectively.

The excellent agreement between the model fit of $\Delta C(t)$ (Eq. 3) and the actually

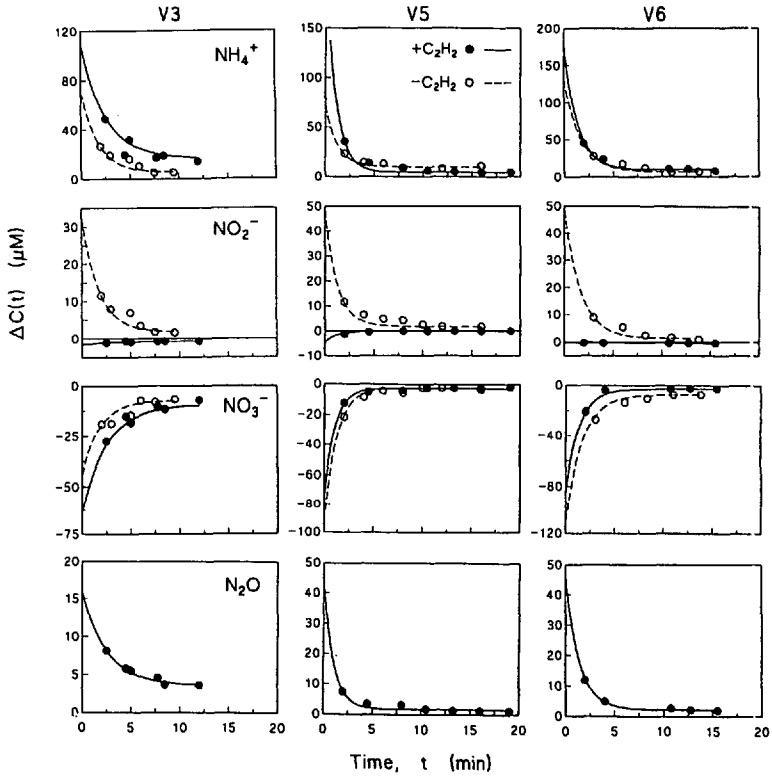

Figure 4. Time-specific excurrent DIN (NH_4^+, NO_2^-, NO_3^-, N_2O-N) concentration pattern during ventilation in 3 *Nereis virens* V-cores (V3, V5, V6). The concentration is presented as $\Delta C(t)$, i.e. the difference between excurrent and incurrent concentrations. The measured values are indicated by open ($-C_2H_2$) and closed ($+C_2H_2$) symbols. The model (Eq. 3) output is represented by broken ($-C_2H_2$) and solid ($+C_2H_2$) curves. Positive values indicate flux out of the burrow.

measured data, as shown in Figure 4, justifies the use of the present model to estimate burrow solute fluxes. In the few cases where the C_o estimate of NO_3^- turned out slightly negative, the concentration was set to $C_o = 0$. The time-specific flux of a solute into or out of a single burrow system ($B(t)$, nmol min^{-1}) during ventilation periods can thus be found by relating the change in solute concentration during water passage to the corresponding ventilation rate:

$$B(t) = (C_{ex}(t) - C_{in})V_w = \Delta C(t)V_w. \tag{8}$$

The total solute mass (μmol) exchanged by a burrow system (burrow + worm) with the overlying water during an entire ventilation period is:

$$M_B = \int_0^{t^*} B(t)\,dt = (C_{ex}^{ss} - C_{in})V_w t^* + (C_o - C_{ex}^{ss})\tau V_w(1 - e^{-t^*/\tau}) \tag{9}$$

Table 2. A. Total DIN exchange between a nereid burrow and overlying water in V-core experiments derived from Eq. 10. B. Rates of DIN exchange across the wall of *N. virens* burrows per m^2 wall area estimated during ventilation periods from Eq. 7. Values are presented as mean (S.D.) of 3 V-cores at each treatment. Negative values denote uptake by the burrow wall.

Treatment	NH_4^+	NO_2^-	NO_3^-	N_2O
A.		J^B (nmol N h^{-1})		
$-C_2H_2$	790 (160)	300 (20)	−680 (200)	—
$+C_2H_2$	1320 (190)	−30 (6)	−600 (100)	270 (60)
B.		J^w (μmol N m^{-2} h^{-1})		
$-C_2H_2$	190 (170)	91 (17)	−330 (140)	—
$+C_2H_2$	200 (110)	−23 (6)	−260 (97)	120 (29)

where $t^* \approx t_f$ (final sample during ventilation period). Since Eq. (9) incorporates C_o (solute concentration at $t = 0$), M_B represents the total net mass exchanged by the burrow due to reactions during both ventilation and rest periods.

If D_v is the fractional time a burrow is ventilated and $\bar{t}^*$ is the average duration of a ventilation period (h), then the total burrow solute flux (nmol h^{-1}) *normalized to both ventilation and rest time periods is:*

$$J^B = M_B D_v / \bar{t}^* \tag{10}$$

Rates. Table 2 shows the estimated total DIN exchange between a burrow and overlying water (J^B; Eq. 10) and the estimated steady state DIN flux across the burrow wall during active ventilation (J^w; Eq. 7). The former estimate is the average of both ventilation and rest periods, and in the case of NH_4^+ it also includes excretion by the worm (R_{nv}).

The total NH_4^+ exchange between a burrow and overlying water ($J^B_{NH_4}$) almost doubled ($\sim 1.7\times$) in the presence of C_2H_2, i.e. from 790 to 1320 nmol h^{-1} (Table 2A). However, much of this was apparently caused by changes in worm excretion. When corrected for contribution by *N. virens,* about 95 ($-C_2H_2$) and 308 ($+C_2H_2$) nmol h^{-1} of the total NH_4^+ flux appeared to originate from burrow walls. The estimated steady state NH_4^+ fluxes across the burrow wall during ventilation alone ($J^w_{NH_4}$), on the other hand, were similar both with and without C_2H_2 additions (Table 2B). The uncertainty in excretion estimates and individual burrow variability (high S.D.) prevents resolution of this apparent discrepancy.

The steady state exchange of oxidized nitrogen across the burrow wall during ventilation (J^w) showed the same general trend as the total exchange (J^B) (Table 2). Although the rate of NO_3^- uptake alone was similar both with and without C_2H_2, the shift from high NO_2^- release ($-C_2H_2$) to low NO_2^- uptake ($+C_2H_2$) resulted in an increase in total oxidized nitrogen ($NO_2^- + NO_3^-$) uptake during C_2H_2 exposure. The NO_2^- release observed in the absence of C_2H_2 was equivalent to 27–44% of the

Table 3. Rates of DIN exchange between sediment and overlying water in bulk-cores with added nereid worms (875 *N. virens* m^{-2}) and defaunated controls. The total exchange by 875 individual burrows m^{-2} estimated from the V-core exchange model (Eq. 10) is presented for comparison. Mean (S.D.) for 3 parallel cores are indicated. Negative values denote uptake by the sediment.

Treatment	J_{NH_4}	J_{NO_2}	J_{NO_3}	J_{N_2O}
		(μmol N $m^{-2}h^{-1}$)		
$-C_2H_2$				
+*N. virens*	360 (100)	49 (3)	−450 (27)	—
control	120 (26)	27 (2)	110 (32)	—
burrows	690 (140)	260 (16)	−590 (180)	—
$+C_2H_2$				
+*N. virens*	1260 (390)	−37 (25)	−1050 (110)	730 (370)
control	510 (160)	−12 (9)	−280 (170)	250 (230)
burrows	1160 (160)	−26 (5)	−520 (91)	240 (53)

measured NO_3^- uptake. A net burrow uptake of $NO_2^- + NO_3^-$ in the absence of C_2H_2 indicates that $NO_2^- + NO_3^-$ reduction predominated over nitrification. Denitrification ($J^w_{N_2O}$, $J^B_{N_2O}$) apparently accounted for 41–43% of the total burrow $NO_2^- + NO_3^-$ uptake.

c. DIN exchange in bulk-cores. DIN fluxes and denitrification rates obtained from bulk-cores were generally stimulated 2–4 times by the presence of *N. virens* (Table 3). This difference compares reasonably with the individual burrow estimates extrapolated to N = 875 *N. virens* m^{-2} ($N \cdot J^B$) both with and without C_2H_2 (Table 3). However, the extensive NO_2^- efflux in burrows ($-C_2H_2$) was not found in the inhabited bulk-cores. The presence of C_2H_2 induced a stimulation of NH_4^+ release in bulk-cores both with and without *N. virens* by a factor of 3–4. The stimulated NH_4^+ flux in control cores (J_{NH_4}, $+C_2H_2$) was in contrast to the estimated flux across burrow walls, where no stimulation by C_2H_2 was apparent ($J^w_{NH_4}$, Table 2B). The NO_2^- exchange changed direction from efflux to influx both in *N. virens* inhabited and control bulk-cores when exposed to C_2H_2, and NO_3^- uptake increased dramatically (controls without C_2H_2 released NO_3^-). Denitrification was stimulated 3-fold by the presence of *N. virens,* but comprised a lower fraction, 67%, of the $NO_2^- + NO_3^-$ uptake than in defaunated controls, 87%.

Unlike V-cores and *N. virens* inhabited bulk-cores, a net release of total oxidized nitrogen, $NO_2^- + NO_3^-$, was observed in control cores without C_2H_2 (Table 3). This clearly demonstrates a dominance of nitrification over $NO_2^- + NO_3^-$ reduction in surface sediment. Inhibition of nitrification by C_2H_2 resulted in a shift to $NO_2^- + NO_3^-$ uptake which quantitatively corresponded to the increase in NH_4^+ release (i.e. ~430 and 390 μmol m^{-2} h^{-1}, respectively).

4. Discussion

The V-core design (Fig. 1) is well suited to quantify ventilation patterns and provides a unique opportunity to model DIN flux rates in individual *Nereis virens* burrows as the influence of the upper sediment surface virtually is eliminated. The exchange-model (Eq. 1–10; Fig. 3) shows an excellent fit to the temporal DIN concentration pattern in samples taken directly from the excurrent water during ventilation periods (Fig. 4). The approximation in the model that ventilation rate is constant throughout a ventilation period does not impose any serious bias in the model output compared to the measured values. The ventilation rate (V_w) can be applied to the model as a time-specific function (i.e. an exponential decrease to a constant level during ventilation periods), but not much is gained by the added complexity given other uncertainties. The primary assumptions, that burrow wall solute flux (J^w) and worm excretion (R_{nv}) are constant during ventilation periods are the best approximations available, as nothing is known about the detailed temporal pattern of these fluxes in ventilated burrows. The flux calculations are sensitive to changes in the parameters C_o and τ, where the former represents the intercept and the latter the slope of the model output (Fig. 4). The accuracy and precision of C_o estimates, which is extrapolated from the measured data (Eq. 6), are dependent on very early initial samplings during ventilation periods and on the assumption of a nearly constant V_w, J^w, and R_{nv}. The generally high turnover of burrow water ($\tau = 1-2$ min^{-1}), which is responsible for a rapid initial dilution during ventilation, emphasizes the need for very early initial samplings. Short-term (minutes) steady state fluxes in a nereid burrow are usually approached at the very end of ventilation periods (Figs. 2–4).

Diffusion-reaction processes involving DIN in bioturbated sediments are generally very sensitive to the number or spacing of burrows (Aller, 1980; 1988). Any single infaunal burrow will interact with the chemistry of surrounding sediment in a radius which is roughly half the distance between any two nearly identical burrows. The present flux estimates of, for example, components which are influenced by sedimentary NH_4^+ sources, are therefore strictly valid for burrows surrounded by a layer of sediment (half inter-burrow distance) which is equivalent to the radius of V-cores (1.35 cm). In other words, the individual burrow flux data presented here are appropriate for a worm density in this sediment of 875 m^{-2} and become less accurate as neighboring burrows of similar size become either closer or farther apart.

a. Nitrogen transformation processes. The diagram in Figure 5 shows the major nitrogen transforming processes (R) and fluxes (J) occurring in NO_3^- rich bioturbated sediments. The diagram represents worm+sediment systems, but is also valid for any nonbioturbated surface sediment by excluding the direct worm contribution (R_{nv}). Each of the DIN fluxes presented in Tables 2 and 3 are the net result of several processes: mineralization (R_{nv}, $R^1_{NH_4}$), nitrification ($R^1_{NO_2}$, R_{NO_3}), nitrate reduction

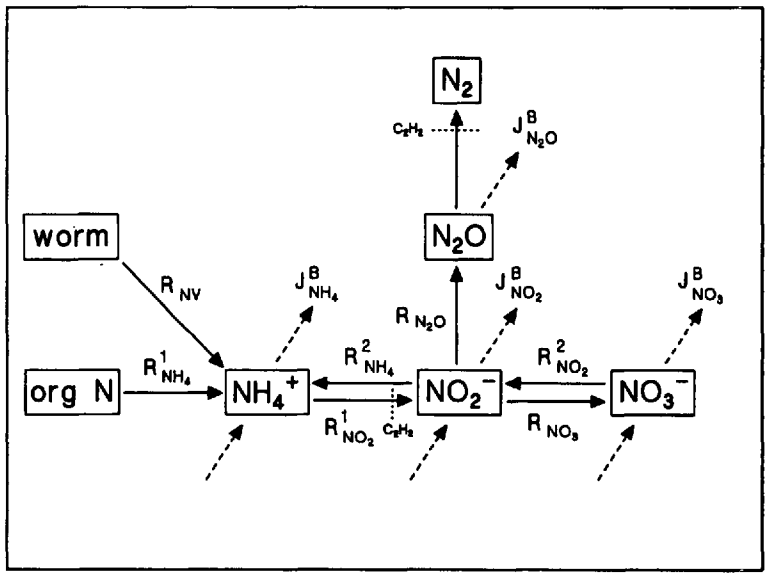

Figure 5. Diagram showing the most important nitrogen transforming processes in a nereid burrow. Boxes represent the various forms of nitrogen involved. Solid arrows (R_z) indicate the microbial processes occurring in sediments (except R_{nv} which is worm excretion). Broken arrows (J^B) symbolize the DIN flux into or out of burrows mediated by the ventilation current. The dotted lines (C_2H_2) indicate the processes blocked by C_2H_2.

($R^2_{NO_2}$), and nitrite reduction ($R^2_{NH_4}$, R_{N_2O}). While C_2H_2 inhibits ammonium oxidation ($R^1_{NO_2}$) and nitrous oxide reduction (Yoshinari and Knowles, 1976; Hynes and Knowles, 1978; Payne, 1984), it is less obvious if and how the other processes are affected either directly or indirectly by this compound. Nitrate and nitrite reducing processes ($R^2_{NO_2}$, $R^2_{NH_4}$, R_{N_2O}) are expected to be independent of C_2H_2 (Kaspar, 1983; Payne, 1984). Thus, the relatively high NO_3^- concentrations ($\sim$100 μM) in the overlying water should counteract the lack of NH_4^+ oxidation and provide the NO_3^- reducing bacteria with an ample supply of oxidant. In strictly quantitative terms, however, it is not known whether this assumption is fully met. A recent study on freshwater sediments indicates that denitrification is underestimated when nitrification is blocked by C_2H_2 even at high overlying water concentrations of NO_3^- (S. Seitzinger, L. P. Nielsen and J. Caffrey, pers. comm.). Despite the stimulation of worm excretion (R_{nv}) by C_2H_2, microbial NH_4^+ production ($R^1_{NH_4}$) is expected to remain unaffected by the presence of C_2H_2 (Payne, 1984; Blackburn 1986). Based on these assumptions the measured and estimated DIN fluxes may be outlined as follows:

$$J^-_{NH_4} = R^1_{NH_4} + R^2_{NH_4} + R^-_{nv} - R^1_{NO_2} \qquad \text{(a)}$$

Table 4. Estimated values of ammonium oxidation ($R^1_{NO_2}$; Eq. h-i) and denitrification (R_{N_2O}; Eq. c) in bulk cores with *Nereis virens,* in control bulk cores, in V-cores (J^B), and in burrow walls (J^w). The values from V-cores (J^B) are presented both as normalized to m^{-2} upper surface sediment (875 *N. virens* m^{-2}) and as burrow rates per m^2 wall area. The estimates from Eq. h are corrected for worm excretion. Values are given as μmol $m^{-2}h^{-1}$.

Incubation series	$R^1_{NO_2}$		R_{N_2O}
	h	i	
Bulk-cores:			
+*N.virens*	620	690	730
control	390	430	250
difference	230	270	480
V-cores:			
J^B (875 m^{-2})	190	220	240
J^B (wall)	51	62	68
J^w (wall)	10	44	120

$$J^+_{NH_4} = R^1_{NH_4} + R^2_{NH_4} + R^+_{nv} \quad \text{(b)}$$

$$J_{N_2O} = R_{N_2O} \quad \text{(c)}$$

$$J^-_{NO_2} = R^1_{NO_2} + R^2_{NO_2} - R^-_{NO_3} - R_{N_2O} - R^2_{NH_4} \quad \text{(d)}$$

$$J^+_{NO_2} = R^2_{NO_2} - R^+_{NO_3} - R_{N_2O} - R^2_{NH_4} \quad \text{(e)}$$

$$J^-_{NO_3} = R^-_{NO_3} - R^2_{NO_2} \quad \text{(f)}$$

$$J^+_{NO_3} = R^+_{NO_3} - R^2_{NO_2} \quad \text{(g)}$$

where the superscripts + and − denote rates determined with and without C_2H_2 exposure. The term R_{nv} is only valid in the cases where worm excretion is included in the fluxes. Denitrification is represented by Eq. c (Table 4). Two independent estimates of ammonium oxidation ($R^1_{NO_2}$) may also be derived from Eqs. d–g according to:

$$R^1_{NO_2} = J^+_{NH_4} - J^-_{NH_4} - R^+_{nv} + R^-_{nv} \quad \text{(h)}$$

$$R^1_{NO_2} = J^-_{NO_3} + J^-_{NO_2} - J^+_{NO_3} - J^+_{NO_2}. \quad \text{(i)}$$

The results of Eqs. h and i (Table 4) based on the present data show good agreement.

b. DIN exchange in individual burrows. The NH_4^+ release from burrows ($J^B_{NH_4}$, Table 2A) is caused by mineralization ($R^1_{NH_4}$) and NO_2^- production/reduction ($R^1_{NO_2}$, $R^2_{NH_4}$) processes in the surrounding sediment, radial diffusion across the burrow wall, and excretion by the worms (R_{nv}) (Aller, 1988; Kristensen, 1988a). Excretion is responsi-

ble for about 80% of the NH_4^+ release from nereid burrows. This is within the range found in previous studies on *N. virens* burrow systems (Henriksen *et al.*, 1983; Kristensen, 1984; 1985). The doubling of NH_4^+ release by addition of C_2H_2 is partly due to C_2H_2-induced stimulation of NH_4^+ excretion by *N. virens* and partly due to inhibition of NH_4^+ oxidation ($R^1_{NO_2}$). Although no influence on the ventilation pattern was found (Table 1), NH_4^+ excretion of *N. virens* increased about 50% by the presence of C_2H_2. The exact physiological mechanisms of this C_2H_2 effect are not presently known, but the simultaneous 100% increase in O_2 uptake indicates that the increase in NH_4^+ excretion is metabolically derived. It is important to consider this effect in studies on NH_4^+ fluxes in C_2H_2 inhibited sediments containing macrofaunal components.

The shift in NO_2^- flux ($J^B_{NO_2}$) from a high release in uninhibited burrows to a low uptake with C_2H_2, suggests that NO_2^- is the major normal product of nitrification in these burrows. Thus, the two estimates of burrow nitrification rate (Eqs. h–i; Table 4) are comparable to the measured NO_2^- release ($J^B_{NO_2}$, Table 2A). Nitrification induced NO_2^- production can only be completely verified when nitrite oxidation (R_{NO_3}) is also determined explicitly, because another possible source of NO_2^- is via NO_3^- reduction. If the change in NO_2^- flux ($J^B_{NO_2}$) is due to C_2H_2 effects on NH_4^+ oxidation ($R^1_{NO_2}$) alone, then all other processes involving NO_2^- ($R^2_{NO_2}$, R_{NO_3}, R_{N_2O}, $R^2_{NH_4}$) must remain unaffected by C_2H_2. As mentioned earlier, the high concentration of NO_3^- in the overlying water may render NO_3^- reduction ($R^2_{NO_2}$) and NO_2^- reduction ($R^2_{NH_4}$, R_{N_2O}) relatively independent of C_2H_2. Since the flux of NO_3^- ($J^B_{NO_3}$) is found to be constant (Table 2A) and $R^2_{NO_2}$ is assumed constant, then, according to Eqs. f–g, NO_2^- oxidation (R_{NO_3}) should also be constant. Furthermore, previous V-core experiments have shown that NO_2^- release in uninhibited cores is independent of NO_3^- reduction at NO_3^- concentrations from 1 to 60 μM in the overlying water (Kristensen, unpublished data).

Under fully aerobic conditions where NH_4^+ oxidation is the rate limiting step of nitrification, no NO_2^- accumulation generally occurs (Kaplan, 1983). However, O_2 limitation of nitrite oxidizers is initiated at relatively high O_2 concentrations, 95 μM (K_m = 62 μM), whereas NH_4^+ oxidizers remain active down to very low O_2 levels, 33 μM (K_m = 16 μM) (Helder and De Vries, 1983; Downes, 1988). The relatively low ventilation activity, high burrow surface to volume ratio combined with the radial diffusion geometry and (in this study) high temperature promotes low O_2 concentrations and penetration depth in burrows. Kristensen (1989) found that the O_2 concentration in burrows of *N. virens,* maintained at 16°C, is below 125 μM for more than 80% of the time. Accordingly, the overall microenvironment in nereid burrows seems to favor growth of NH_4^+ oxidizers relative to NO_2^- oxidizers.

The rate of NO_3^- reduction exceeds nitrification at the present incurrent NO_3^- concentration (Tables 3 and 4). This is consistent with earlier findings, that NO_3^- is only released from *N. virens* burrows at low ambient concentrations ($<$10–15 μM;

Kristensen, 1984). The general low O_2 availability in burrows promotes advantageous conditions for NO_3^- reducers compared to nitrifiers (Henriksen *et al.,* 1983). The rate of NO_2^- + NO_3^- uptake ($J^B_{NO_2}$ + $J^B_{NO_3}$) observed after C_2H_2 addition represents the total NO_2^- + NO_3^- reduction (R_{N_2O} + $R^2_{NH_4}$) in burrows. The associated N_2O release ($J^B_{N_2O}$) comprises about 40% of the net DIN release from burrows indicating the quantitative importance of burrow wall denitrification. Denitrification accounts for 43% of the NO_2^- + NO_3^- uptake. The remaining NO_2^- + NO_3^- uptake is due to other reducing pathways, NO_3^- ammonification ($R^2_{NH_4}$) and assimilation during bacterial growth (Koike and Sørensen, 1988), or is a result of an inefficient C_2H_2 inhibition of N_2O reduction. *N. virens* burrow walls are known to support a considerable potential for both denitrification and NO_3^- ammonification (Kristensen *et al.,* 1985). Nothing is presently known about assimilatory NO_3^- uptake in burrows, but it is normally assumed that this process is insignificant when NH_4^+ concentrations are high (Brown *et al.,* 1975) such as in sediments. Estimates of NO_3^- ammonification by subtraction of denitrification from the total NO_2^- + NO_3^- reduction in C_2H_2 inhibited cores may, however, tend to overestimate the true rate (cf. Jørgensen, 1989). Reduced efficacy of the C_2H_2 block in the presence of H_2S and at NO_3^- concentrations below a certain threshold (5–10 μM) may result in underestimated denitrification rates (Sørensen *et al.,* 1987; Slater and Capone, 1989). In the present study NO_3^- concentrations are relatively high and there was no obvious free H_2S. Recent studies have also shown that significant N_2O reduction can occur in C_2H_2 treated cores at high NO_3^- concentrations in the overlying water, i.e 100 μM or higher (Christensen *et al.,* 1989; Nielsen, *et al.,* 1990). It is thus not known to what extent, if at all, denitrification is underestimated in the present study.

c. DIN exchange in bulk-cores; comparison with individual burrows. The influence of *N. virens* on DIN exchange in bulk-cores (2–4 times increase) is in agreement with previous reports on this and other infaunal species (Henriksen *et al.,* 1980; 1983; Aller and Yingst, 1985; Kristensen, 1985; Matisoff *et al.,* 1985; Kristensen and Blackburn, 1987; Andersen and Kristensen, 1988). The DIN flux patterns in bulk-cores also correspond well to those extrapolated from individual burrows, although the difference in fluxes between *N. virens* inhabited and control bulk-cores is lower for NH_4^+ and NO_2^- ($-C_2H_2$) and higher for NO_3^- ($+C_2H_2$) and N_2O than in V-cores (Table 3). In both core systems, the release of NH_4^+ is stimulated 2–4 times and the NO_2^- flux changes direction from release to uptake when C_2H_2 is added. The difference between nitrification estimates ($R^1_{NO_2}$) in bulk-cores with and without *N. virens* (i.e. burrow nitrification) is similar to the burrow nitrification estimate from V-cores (Table 4). For denitrification, however, the burrow contribution estimated from bulk-cores is higher than the measured rate in V-core burrows.

In accordance with the models of Aller (1988), the presence of *N. virens* profoundly alters the balance between nitrification and NO_3^- reduction compared to the

non-bioturbated sediment. Thus, $NO_2^- + NO_3^-$ is released from control cores and is taken up in *N. virens* inhabited cores in the absence of C_2H_2 (Table 3). In *N. virens* inhabited bulk-cores, the measured denitrification rates are almost similar to the nitrification estimates, while in defaunated controls, denitrification only accounts for 60% of the estimated nitrification. This indicates that total sediment denitrification is stimulated more than total nitrification by the presence of *N. virens* at natural abundances. In C_2H_2 inhibited bulk-cores, where no nitrification occurred, total denitrification and $NO_2^- + NO_3^-$ uptake is highly stimulated in the presence of *N. virens* (by a factor of 3) indicating that burrow walls are active sites of NO_3^- reducing activity. Kristensen *et al.* (1985) found that the capacity (mass/volume/time) for denitrification and NO_3^- reduction in *N. virens* burrow walls is similar to the sediment surface, implying that the enhanced NO_3^- availability due to burrow ventilation and the radial diffusion geometry around burrows are primarily responsible for the increased NO_3^- respiration in these sediments. Denitrification comprises 67 and 87% of the $NO_2^- + NO_3^-$ uptake in *N. virens* cores and controls, respectively. This is within the range usually found in coastal sediments using similar techniques (Kaspar, 1983; Goeyens *et al.,* 1987; Koike and Sørensen, 1988).

d. The importance of burrows in sediment nitrogen cycling. Burrow structures are known as important sites of both nitrification and dissimilatory NO_3^- reduction in coastal marine sediments (Henriksen *et al.,* 1980; Sayama and Kurihara, 1983; Kristensen *et al.,* 1985; Kristensen and Blackburn, 1987). However, only few have attempted to quantify the role of burrows in these processes. Under the present experimental conditions, *N. virens* burrows are responsible for 37 and 66% of the total sediment nitrification and denitrification, respectively (estimated from bulk-core values; Table 4). Henriksen *et al.* (1980) found values of 35 and 38% in an estuarine Limfjord sediment (3–4 μM NO_3^- in overlying water and 2000 *N. virens* m^{-2} at 10°C). A direct comparison of these estimates is, in addition to differences in temperature and worm density, prevented by the strong dependence of NO_3^- reduction activity in burrows on overlying water NO_3^- concentration (Henriksen *et al.,* 1983; Kristensen, 1984). Based on potential rate measurements, Kristensen *et al.* (1985) estimated from a two-dimensional model that burrows account for about 35 and 52% of the total sediment nitrification and denitrification, respectively, in Norsminde Fjord. Oxygen penetration depths into burrow walls and surface sediment of 1 and 5 mm, respectively, were assumed at a *N. virens* density of 600 m^{-2}. The more accurate radial model of Aller (1988) estimates higher but comparable values of 41 and 66%, respectively.

The present study clearly demonstrates and emphasizes the overall importance of ventilated burrow structures in the sediment nitrogen cycle. *Nereis virens* stimulates both the microbial transformation and the net fluxes across the sediment-water interface of all DIN compounds by a factor of 2–4. The previously unrecognized high

NO_2^- release from burrows is suggested to be associated with nitrification rather than nitrate reduction. The ratio of total denitrification to total nitrification in the sediment is almost doubled from 0.61 to 1.11 by the presence of 875 *N. virens* m^{-2}. Under natural conditions with a mixed infaunal assemblage this ratio may increase, decrease or even remain unaffected depending on the actual abundance and species composition of animals present (Henriksen *et al.,* 1983; Aller, 1988). Various infaunal species may differ in impact on nitrogen transformations in sediments, but they are all, as found for *N. virens,* probably responsible for net nitrogen removal (as N_2) by the simultaneous stimulation of both nitrification and denitrification.

Acknowledgments. We wish to thank Torben Kjær Andersen for his invaluable help in planning and execution of this work. Thanks are also due to a green olive (*Olivia festivitas*) which determined the authorship. R.C.A. was supported by NSF grant OCE-9001397.

REFERENCES

Aller, J. Y. and R. C. Aller. 1986. Evidence for localized enhancement of biological activity associated with tube and burrow structures in deep-sea sediments at the Hebble site, Western North Atlantic. Deep-Sea Res., *33,* 755–790.

Aller, R. C. 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. Geochim. Cosmochim. Acta, *44,* 1955–1965.

—— 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water, *in* Animal-Sediment Relations, P. L. McCall and M. J. S. Tevesz, eds., Plenum Press, New York, 53–102.

—— 1983. The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. J. Mar. Res., *41,* 299–322.

—— 1988. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures, *in* Nitrogen Cycling in Coastal Marine Environments, T. H. Blackburn and J. Sørensen, eds., John Wiley & Sons, Chichester, 301–338.

Aller, R. C. and J. Y. Yingst. 1978. Biogeochemistry of tube-dwellings: a study of the sedentary polychaete *Amphitrite ornata* (Leidy). J. Mar. Res., *36,* 201–254.

—— 1985. Effects of the marine deposit-feeders *Heteromastus filiformis, Macoma balthica* and *Tellina texana* on averaged sedimentary solute transport, reaction rates and microbial distribution. J. Mar. Res., *43,* 615–645.

Aller, R. C., J. Y. Yingst and W. J. Ullman. 1983. Comparative biogeochemistry of water in intertidal *Onuphis* (polychaete) and *Upogebia* (crustacea) burrows: temporal patterns and causes. J. Mar. Res., *41,* 571–604.

Andersen, F. Ø. and E. Kristensen. 1988. The influence of macrofauna on estuarine benthic community metabolism: a microcosm study. Mar. Biol., *99,* 591–603.

Andersen, T. K., M. H. Jensen and J. Sørensen. 1984. Diurnal variation of nitrogen cycling in coastal, marine sediments. I. Denitrification. Mar. Biol., *83,* 171–176.

Armstrong, F. A., C. R. Stearns and J. D. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res., *14,* 381–389.

Balderston, W. L., B. Sherr and W. J. Payne. 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus.* Appl. Environm. Microbiol., *31,* 504–508.

Blackburn, T. H. 1986. Nitrogen cycle in marine sediments. Ophelia, *26,* 65–76.

Brown, C. M., D. S. MacDonald-Brown and S. O. Stanley. 1975. Inorganic nitrogen metabolism in a marine Pseudomonad. Mar. Biol., *31,* 7–13.

Christensen, P. B., L. P. Nielsen, N. P. Revsbech and J. Sørensen. 1989. Microzonation of denitrification activity in stream sediments as studied with a combined oxygen and nitrous oxide microsensor. Appl. Environm. Microbiol., *55,* 1234–1241.

Downes, M. T. 1988. Aquatic nitrogen transformations at low oxygen concentrations. Appl. Environm. Microbiol., *54,* 172–175.

Goeyens, L., R. T. P. De Vries, J. F. Bakker and W. Helder. 1987. An experiment on the relative importance of denitrification, nitrate reduction and ammonification in coastal marine sediment. Neth. J. Sea Res., *21,* 171–175.

Helder, W. and R. T. P. De Vries. 1983. Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard estuary). Neth. J. Sea Res., *17,* 1–18.

Henriksen, K., J. I. Hansen and T. H. Blackburn. 1980. The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. Ophelia suppl., *1,* 249–256.

Henriksen, K., M. B. Rasmussen and A. Jensen. 1983. Effect of bioturbation on microbial nitrogen transformations in the sediment and fluxes of ammonium and nitrate to the overlaying water. Ecol. Bull. (Stockholm), *35,* 193–205.

Hylleberg, J. and K. Henriksen. 1980. The central role of bioturbation in sediment mineralization and element recycling. Ophelia suppl., *1,* 1–16.

Hynes, R. K. and R. Knowles. 1978. Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europea.* FEMS Microbiol. Lett., *4,* 319–321.

Jensen, M. H., T. K. Andersen and J. Sørensen. 1988. Denitrification in coastal bay sediment: regional and seasonal variation in Aarhus Bight, Denmark. Mar. Ecol. Prog. Ser., *48,* 155–162.

Jørgensen, B. B. and N. P. Revsbech. 1985. Diffusive boundary layers and oxygen uptake of sediments and detritus. Limnol. Oceanogr., *30,* 111–122.

Jørgensen, B. B. and J. Sørensen. 1985. Seasonal cycles of O_2, NO_3^- and SO_4^{2-} reduction in estuarine sediments: the significance of an NO_3^- reduction maximum in spring. Mar. Ecol. Prog. Ser., *24,* 65–74.

Jørgensen, K. S. 1989. Annual pattern of denitrification and nitrate ammonification in estuarine sediment. Appl. Environm. Microbiol., *55,* 1125–1134.

Jørgensen, K. S. and J. Sørensen. 1988. Two annual maxima of nitrate reduction and denitrification in estuarine sediment (Norsminde Fjord, Denmark). Mar. Ecol. Prog. Ser., *48,* 147–154.

Kaplan, W. A. 1983. Nitrification, *in* Nitrogen in the Marine Environment, E. J. Carpenter and D. G. Capone, eds., Academic Press, New York, 139–190.

Kaspar, H. F. 1983. Denitrification, nitrate reduction to ammonium and inorganic nitrogen pools in intertidal sediments. Mar. Biol., *74,* 133–139.

Koike, I. and J. Sørensen. 1988. Nitrate reduction and denitrification in marine sediments, *in* Nitrogen Cycling in Coastal Marine Environments, T. H. Blackburn and J. Sørensen, eds., John Wiley & Sons, Chichester, 251–273.

Krantzberg, G. 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: a review. Environm. Pollut., *39A,* 99–122.

Kristensen, E. 1984. Effect of natural concentrations on nutrient exchange between a polychaete burrow in estuarine sediment and the overlying water. J. Exp. Mar. Biol. Ecol., *75,* 171–190.

—— 1985. Oxygen and inorganic nitrogen exchange in a *Nereis virens* (Polychaeta) bioturbated sediment-water system. J. Coastal Res., *1,* 109–116.

—— 1988a. Benthic fauna and biogeochemical processes in marine sediments: microbial activities and fluxes, *in* Nitrogen Cycling in Coastal Marine Environments, T. H. Blackburn and J. Sørensen, eds., John Wiley & Sons, Chichester, 275–299.

—— 1988b. Factors influencing the distribution of nereid polychaetes in Danish coastal waters. Ophelia, *29,* 127–140.

—— 1989. Oxygen and carbon dioxide exchange in the polychaete *Nereis virens:* influence of ventilation and starvation. Mar. Biol., *101,* 381–388.

Kristensen, E. and T. H. Blackburn. 1987. The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. J. Mar. Res., *45,* 231–257.

Kristensen, E., M. H. Jensen and T. K. Andersen. 1985. The impact of polychaete (*Nereis virens* Sars) burrows on nitrification and nitrate reduction in estuarine sediments. J. Exp. Mar. Biol. Ecol., *85,* 75–91.

Markham, A. E. and K. A. Kobe. 1941. The solubility of carbon dioxide and nitrous oxide in aqueous salt solution. J. Am. Chem. Soc., *63,* 449–454.

Matisoff, G., J. B. Fisher and S. Matis. 1985. Effects of benthic macroinvertebrates on the exchange of solutes between sediments and freshwater. Hydrobiologia, *122,* 19–33.

Nielsen, L. P., P. B. Christensen, N. P. Revsbech and J. Sørensen. 1990. Denitrification and photosynthesis in stream sediment studied with microsensor and whole core techniques. Limnol. Oceanogr., *35,* 1135–1144.

Payne, W. J. 1984. Influence of acetylene on microbial and enzymatic assays. J. Microbiol. Meth., *2,* 117–133.

Reichardt, W. 1986. Polychaete tube walls as zonated microhabitats for marine bacteria. Actes Colloq. IFREMER, *3,* 415–425.

—— 1988. Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. Mar. Ecol. Prog. Ser., *44,* 149–158.

Sayama, M. and Y. Kurihara. 1983. Relationship between burrowing activity of the polychateous annelid *Neanthes japonica* (Izuka) and nitrification-denitrification processes in the sediments. J. Exp. Mar. Biol. Ecol., *72,* 233–241.

Slater, J. M. and D. G. Capone. 1989. Nitrate requirement for acetylene inhibition of nitrous oxide reduction in marine sediments. Microb. Ecol., *19,* 143–157.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr., *14,* 799–801.

Sørensen, J. 1978. Denitrification rates in marine sediments as measured by the acetylene inhibition technique. Appl. Environm. Microbiol., *36,* 139–143.

Sørensen, J., L. K. Rasmussen and I. Koike. 1987. Micromolar sulfide concentrations alleviate acetylene blockage of nitrous oxide reduction by denitrifying *Pseudomonas fluorescens.* Can. J. Microbiol., *33,* 1001–1005.

Walter, H. M., D. R. Keeney and I. R. Fillery. 1979. Inhibition of nitrification by acetylene. Soil. Sci. Soc. Am. J., *43,* 195–196.

Weiss, R. F. and B. A. Price. 1980. Nitrous oxide solubility in water and seawater. Mar. Chem., *8,* 347–359.

Winston, J. E. and F. E. Anderson. 1971. Bioturbation of sediments in a northern temperate estuary. Mar. Geol., *10,* 39–49.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Comm., *69,* 705–710.

Received: *19 December, 1989;* revised: *19 March, 1991.*