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Measurement of in Situ Rates of Nitrification in Sediment

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Abstract. A method has been developed for the measurement of nitrification rates in intact sediment cores without disturbing the concentration gradients of oxygen and ammonium. N-serve (2-chloro-6-trichloromethyl-pyridine), a specific inhibitor of the autotrophic ammonium oxidation, was injected into a 0–2 cm surface layer of the sediment (20 ppm) and added to the water column of sediment cores (5 ppm). N-serve in these concentrations was sufficient to inhibit nitrification, but did not change the rate of ammonium production or incorporation in sediment suspensions, which were incubated aerobically and anaerobically. The ammonium accumulation in cores injected with N-serve was thus equal to the amount of ammonium which was oxidized to nitrate in the control cores. Nitrification rates were in the range of 0–3 mmol N m^{-2} d^{-1}.

Introduction

N-serve inhibits specifically the nitrifying bacteria *Nitrosomonas* in oxidizing ammonium to hydroxylamine, the first step in the nitrification process (5). N-serve has been used to measure ^{14}C-bicarbonate incorporation by nitrifying bacteria, and the incorporation rate was related to the rate of ammonium oxidation (2). The sediment was fractionated and incubated aerobically, thus disturbing the oxygen and ammonium gradients, which regulate nitrification rates in the sediment. We have found that high numbers of nitrifying bacteria occur below the zone of oxygen penetration in marine sediments and can almost immediately oxidize ammonium if supplied with oxygen (Hansen, M.Sci. thesis, 1980). Methods in which oxygen is introduced into anoxic parts of the sediment are, therefore, likely to give potential rather than actual nitrification rates. This problem may be avoided by using intact sediment cores, in which near in situ concentration gradients of oxygen and ammonium are maintained during the incubation.

Materials and Methods

Aerobic Incubations

The surface layer (0–2 cm) of a fine-textured sediment (The Limfjorden, 12 m water depth) and a sandy sediment (Kysing Fjord, 1 m water depth) from inner Danish waters were used for aerobic incubations. The

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sediment was preincubated in seawater with stirring for 24 h. Five grams of the homogenized sediment was added to serum bottles (60 ml) containing 50 ml of filtered seawater enriched with NH_4^+ and NO_3^-. Parallel series of samples with different concentrations of N-serve were incubated with vigorous stirring.

The influence of N-serve on ammonium turnover was studied by adding $^{15}NH_4Cl$ (90.4% ^{15}N, VEB Berlin) to the ammonium pool at a concentration of 6.3% ^{15}N. Triplicate samples of each series were stopped at intervals and analyzed for KCl-extractable NH_4^+, NO_x (NO_2^- + NO_3^-) and ^{15}N content of the NH_4^+ pool.

Anaerobic Incubations

The 4–6 cm layers of cores from the same localities were mixed anaerobically in a 1:1 ratio (w/w) with N_2-bubbled seawater and incubated in glass centrifuge tubes, stoppered under nitrogen gas. Parallel series with and without N-serve were incubated. Again, to test any influence of N-serve on ammonium turnover, $^{15}NH_4Cl$ was added to some series to a concentration of 6% ^{15}N in the NH_4^+ pool. Triplicate samples from each series were stopped at timed intervals and analyzed for KCl-extractable NH_4^+ and ^{15}N%.

Procedure for Nitrification Rate Measurements in Intact Sediment Cores

Cores were taken in Plexiglas tubes (2.6 cm i.d.) to a depth of 8 cm either by hand or subsampled from a "Haps" bottom corer (8). Only cores with undisturbed stratification were used, and cores containing larger macrobenthic fauna (e.g., polychaetes and bivalves) were discarded. Twelve cores were used for each assay. The water column was replaced with a known volume of filtered seawater (35 ml) from the same location. N-serve was added to the water column of half of the cores to a concentration of 5 ppm. The tubing of these cores had a vertical series of silicone rubber inserts placed at 5 mm intervals. N-serve was injected in the 0–2 cm layer of the sediment through the inserts, using a 10 μl glass syringe with a thin needle. The injected N-serve (10 μl) was distributed at seven different points in the horizontal plane to minimize diffusion distance ($\leq$5 mm). Final concentration of N-serve in the 0–2 cm layer was 20 ppm (μg/g of wet sediment). The cores were incubated for 3 to 4 days in the dark at in situ temperature with aeration of each water column through a thin needle. This treatment was necessary to maintain in situ oxygen profiles, as oxygen penetraton of the sediment decreased markedly without any turbulence in the water column (9). It also kept NH_4^+ and NO_3^- profiles of the control cores unchanged during incubation. The relatively long incubation time was necessary to obtain significant differences in ammonium concentrations between test and control cores; there was considerable variation between cores.

To assure full inhibition of nitrification during the incubation period, the water column of each core was changed at intervals of 1 or 2 days, depending on the rate of N-serve disappearance (tested by smelling). Fresh N-serve was added to the water column of the N-serve-treated cores. The ammonium concentration of each water column was measured. At the end of the incubation, cores were sectioned in 1 cm intervals and KCl-extractable NH_4^+ was measured. The nitrification rate per area (nmol N cm^{-2} d^{-1}) was calculated from the difference between the means of ammonium content (water column + sediment) for N-serve-treated and control cores.

Photosynthetic Activity of Benthic Microalgae

Photosynthetic O_2-production was measured in light and dark glass bottles in full daylight (20°C). Then 5 g of homogenized surface sediment (0–5 mm) from the shallow sandy station was incubated in filtered seawater for 4 h. Parallel series with different concentrations of N-serve (0, 5, 10, and 20 ppm) were run to test the influence of N-serve on photosynthetic activity of benthic microalgae.

Chemical Assays

NH_4^+, NO_2^-, and NO_3^- were measured by an autoanalyzer (Chemlab Instruments, Ltd., Essex) using the methods of Solórzano (12) and Armstrong et al. (1). The NH_4^+ pool was extracted from the sediment with 1N

KCl (1:1, w/w). The samples were shaken for 10 min at 2000 *g*. An increase in KCl concentration or extraction time did not increase the yield of ammonium.

^{15}N analyses were made on a Statron NOI 4 optical emission ^{15}N analyzer. Preparation and analyses of ammonium samples were done according to the method described by Blackburn (3).

N-serve was "technical grade" (95% N-serve, 5% other chlorinated pyridines) obtained from the Dow Chemical Company Ltd. (Kings Lynn, Norfolk). It was dissolved in acetone (stock solution 100 mg ml^{-1}). Before use, the stock solution was diluted 1:10 with acetone (10 mg ml^{-1}). The possible effect of acetone on nitrogen turnover was tested in aerobic and anaerobic incubations of sediment at concentrations equivalent to the N-serve concentrations used.

For measurements of N-serve concentrations in water column and sediment, N-serve was extracted with a 1:1 mixture of hexane and acetone. The hexane layer was separated and the N-serve concentration measured on a Hewlett Packard gas chromatograph with ECD-detector following the method of Briggs (4).

Oxygen determinations in seawater were done by the method of Strickland and Parsons (13).

Results

Effect of N-serve on Microbial Ammonium Turnover

In aerobic incubations, the difference in ammonium concentration (ΔNH_4^+) between N-serve inhibited (B) and control series (A) equalled the NO_x^- production (ΔNO_x^-) in the control series (Fig. 1). Over a 48-h period, ΔNH_4^+ was 960 nmol N g^{-1} and the nitrate accumulation (ΔNO_x^-) 1010 nmol N g^{-1}. In the N-serve-inhibited series (B), NO_x^- concentration was constant with time.

The consistency between ΔNO_x^- and ΔNH_4^+ indicated that total ammonium production (*d*) and ammonium incorporation into cells (*i*) were unaffected or that both *d* and *i* were equally affected by N-serve. To test this, rates of *d* and *i* for series A and B were calculated from the dilution rate of ^{15}N-NH_4^+ according to the theory described by Blackburn for ammonium turnover in anoxic sediments (3). In the control series, the nitrification rate (*a*), measured as ΔNO_x^-, was added to the rate of ammonium incorporation ($i + a$) in the equation used (Table 1). Although the system was not in equilibrium, with constant rates of *d* and *i* during the incubation period, the rates can be considered near constant during the separate time intervals. There was no significant difference in total ammonium production or incorporation between N-serve-inhibited and control series at the N-serve concentrations used.

In aerobic incubations with different concentrations of N-serve, effective inhibition of NO_x^- production ($\geq$97%) was found down to a concentration of 5 ppm N-serve. This is in agreement with the inhibition of ^{14}C-bicarbonate assimilation in enrichment cultures of nitrifying bacteria from marine sediments (2).

Also in aerobic incubations of sandy sediment with different N-serve concentrations (0, 20, 40 ppm), ΔNH_4^+ equalled ΔNO_x^-, and there was apparently no effect of N-serve on ammonium production or incorporation, even at the highest N-serve concentration used (Fig. 2).

In anaerobic incubations of fine-textured sediment, rates of ammonium turnover were not affected by addition of 50 ppm N-serve (Table 2). Net ammonium production ($d - i$) of anaerobic incubations of sandy sediment with different concentrations of N-serve (0, 25, 50 ppm) also showed no significant difference between control samples and N-serve-treated samples (unreported results).

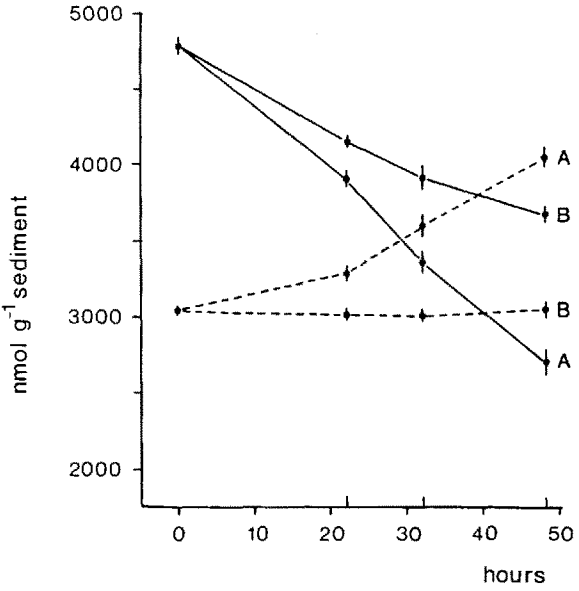

Fig. 1. Concentrations of NH_4^+ (●——●) and NO_x^- (●----●) in sediment samples (The Limfjorden) incubated at 12°C in the dark under aerobic conditions. A Control; B +20 ppm N-serve. SDs of triplicate samples are indicated.

In aerobic and anaerobic incubations of sandy and fine-textured sediment, no effect of the solvent acetone on NO_x^- accumulation (ΔNO_x^-) or net ammonium production ($d - i$) were found at concentrations of 3 to 6 μl acetone per gram of wet sediment (equal to 20–50 ppm N-serve).

Effect of N-serve on Benthic Algae and Macrofauna

No inhibition of the photosynthetic O_2 production of benthic microalgae from the surface layer (0–5 mm) of sandy sediment was observed by the presence of up to 10 ppm

Table 1. Rates of ammonium turnover in aerobic incubations of fine-textured sediment from The Limfjorden (nmol N g^{-1} h^{-1})

	Control (A)			N-serve (20 ppm) (B)		
Time interval (h)	d	i	a_1	d	i	a_2
0–22	8	37	11	7	36	11
23–32	23	44	33	22	46	30
33–48	13	26	28	11	26	26

a_1 = nitrification rate measured as ΔNO_x^-

a_2 = nitrification rate measured as ΔNH_4^+ between A and B.

The following equation was used for calculation of rates of ammonium turnover (3): $\ln R_t = \ln R_0 - d/d - i \times \ln [(d - i)t + P_0]/P_0$. For the control series A, $i + a$ was used instead of i. $R = {}^{15}N\%$ of NH_4^+ (corrected for natural background), d = rate of total ammonium production, i = rate of ammonium incorporation, a = nitrification rate, measured as ΔNO_x^-, and $P = NH_4^+$ concentration.

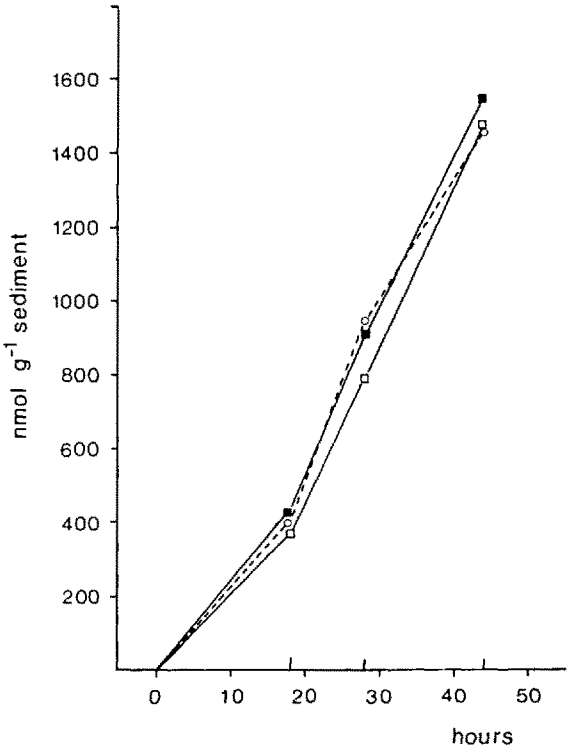

Fig. 2. Sandy sediment (Kysing Fjord) incubated at 14°C in the dark under aerobic conditions with different concentrations of N-serve. Nitrification rates calculated as ΔNO_x^- (o---o) and as ΔNH_4^+ between control and 20 ppm N-serve series (□——□) and between control and 40 ppm N-serve series (■——■).

N-serve, whereas O_2 production was markedly suppressed at a concentration of 20 ppm N-serve. This is in agreement with results obtained by Shattuck and Alexander (11), who found no inhibition of growth at a concentration of 10 ppm N-serve in cultures of different algal groups.

The benthic macrofauna were in general sensitive to N-serve. *Corophium volutator* (Crustaceae) and *Nereis virens* (Polychaeta) could tolerate only 5 ppm N-serve in seawater without any change in ammonium excretion or activity. *Hydrobia ulvae* (Gastropoda), however, were not inhibited by up to 20 ppm N-serve. A sediment concentration of 20 ppm N-serve could be tolerated by all species investigated if the concentration of the water column was 5 ppm.

Table 2. Rates of ammonium turnover in anaerobic incubations of fine-textured sediment from The Limfjorden (nmol N g^{-1} d^{-1})

	Control (A)			N-serve (50 ppm) (B)		
Time interval (h)	*d*	*i*	*d* − *i*	*d*	*i*	*d* − *i*
0–24	38	22	16	39	23	16
24–48	24	7	17	24	7	17

Calculated from the equation listed in Table 1.

Degradation of N-serve in Sediment and Water Column

N-serve was rapidly degraded under reduced conditions and the process is believed to be a chemical hydrolysis (Dow Chemical Company, personal communication). In oxidized sediment, however, N-serve was slowly degraded with a rate depending on temperature. In oxidized sandy sediment no decrease in N-serve concentration was observed after 24 h at 3°C, whereas 70% had disappeared at room temperature. In seawater, N-serve degraded slowly even at room temperature. A decrease of 5% was found after 24 h.

When N-serve was mixed into the sediment, part of it absorbed to organic matter and was probably removed from the active pool. In mud (12% organic matter) only 40–50% of the added N-serve was recovered in the pore water fraction, whereas 80–90% was recovered in pore water from the sandy sediment (3% organic matter). N-serve was easily detected down to a concentration of 1 ppm by its characteristic odor.

Nitrification in Intact Sediment Cores

The N-serve method was used on a variety of marine sediment types from shallow and deeper parts of coastal Danish waters at different seasons. An example is illustrated in Fig. 3.

There was an increase in ammonium concentration in N-serve-inhibited, fine-textured sediment from The Kattegat (17 m water depth) compared to the control. The difference between the test and the control cores represents the ammonium which would have gone to form NO_x^-. Due to diffusion, the increase in ammonium concentration to a depth of 8 cm in the test cores does not imply that nitrification occurred to that depth.

Nitrate in the sediment did not occur below 6 cm. From the two-layered stationary model of Vanderborght and Billen (14), a theoretical nitrate profile can be calculated if the nitrification rate (K_n) and the depth of the nitrification zone (Z_n) in the sediment are known. In Fig. 4, the calculated nitrate profiles for two depths of Z_n (A: 0–5 mm depth, B: 0–10 mm depth) are compared with the actual measured nitrate profile at the Kattegat station. The measured value was used as the nitrification rate (K_n) and converted to rate per volume corresponding to the Z_n value chosen. Other constants were as in the model.

Discussion

In the aerobic incubation experiments, N-serve did not affect the total ammonium production (d), as deduced from the isotope dilution rate. This was also true for the ammonium incorporation rate (i) into bacterial cells, provided that the measured nitrate production in the control series (ΔNO_x^-) was a true estimate of the nitrification rate (a), e.g., if denitrification or nitrate assimilation did not occur during the incubation. N-serve in the concentrations used did not affect denitrification (7). The constant NO_x^- concentration in the N-serve-inhibited series indicated, therefore, that there was a total inhibition of denitrification during oxic incubation. It also indicated that no nitrate assimilation occurred, consistent with bacterial preference of ammonium over nitrate as a nitrogen source (6). It can thus be concluded that ΔNO_x^- (and therefore ΔNO_4^+) can be considered to be a true measure of the nitrification rate, since organic N mineralization (d) and ammonium uptake (i) were unaffected by N-serve. It may similarly be

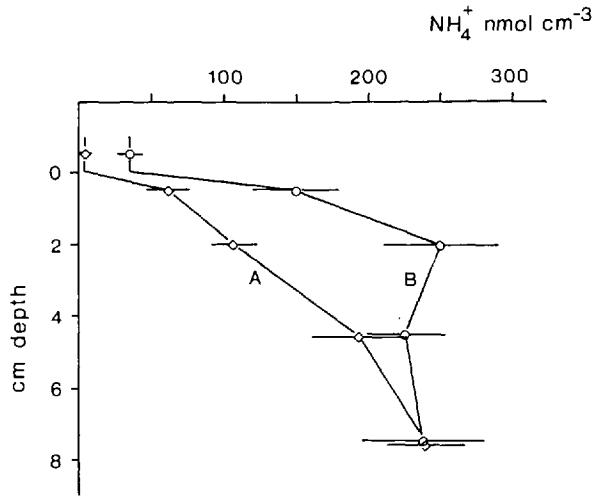

Fig. 3. NH_4^+ concentrations of undisturbed sediment cores from Kattegat (Sept. 1978, 25 m depth) after incubation for 5 days at in situ temperature (13°C). A Control; B + N-serve. SDs of 5 cores are indicated.

concluded that in anaerobic incubations N-serve in concentrations up to 50 ppm did not affect ammonium turnover. The actual concentrations of N-serve at times after the addition were not known, but the data indicate that there was sufficient N-serve to completely inhibit nitrification.

No effect of N-serve on benthic microflora or macrofauna was found at concentrations sufficient to inhibit the nitrification process. This is important, since the death and decomposition of microalgae or macrofauna would give erroneously high ammonium accumulation rates in the N-serve-treated cores. Although the activity and ammonium excretion of macrofauna were not affected, cores containing large animal species (polychaetes and bivalves) could not be used since the ammonium profiles were so different from those of cores which did not contain them. The method demands that all initial ammonium and oxygen gradients in control and test cores are similar.

From the present results, it seems possible to obtain a specific inhibition of the autotrophic nitrification process in intact sediment cores at the N-serve concentrations used. Although N-serve is degraded in the sediment, replacement of the water column with fresh N-serve assures full inhibition during the incubation. The advantage of using intact sediment cores is that concentrations of oxygen and ammonium are kept near in situ conditions during the incubation. The availability of oxygen and ammonium limits nitrification in marine sediments, where pH conditions are almost constant and near optimum. Oxygen penetration in Danish coastal sediments was limited to the upper 2 to 6 mm (10). This restricts nitrifying activity to a narrow zone at the sediment surface and to the thin oxic lining of infauna burrows, but nitrifying bacteria are found deep in the sediment and are almost immediately activated by exposure to oxygen. The introduction of oxygen into the anoxic parts of the sediment during incubation would, therefore, give an overestimate of the actual nitrification rate (2, 14).

The advantages of using in situ incubation conditions are offset by lack of sensitivity, due to natural variation in ammonium profiles, often caused by bioturbation or high rates of ammonium production as in late summer.

Nitrification rates measured by the method in different sediment types from coastal

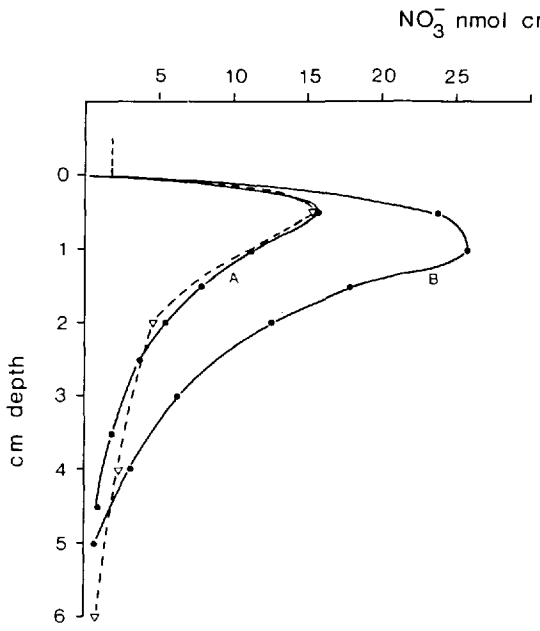

Fig. 4. Measured (▽ ---- ▽) and calculated (●——●) nitrate profiles at the Kattegat station. The following parameters were used for calculation of the nitrate profiles by the model of Vanderborght and Billen (12): Diffusion coefficient $D = 2 \times 10^{-5}$ cm^{-2} s^{-1}. Denitrification rate constant $k_d = 10^{-5}$ s^{-1}. *A* Z_n = 0.5 cm, K_n = 300 nmol N cm^{-3} d^{-1}. *B* Z_n = 1.0 cm, K_n = 150 nmol N cm^{-3} d^{-1}

Danish waters were in the range of 0 to 300 nmol N cm^{-2} d^{-1} (Henriksen, unpublished). The narrow oxygen-containing zone at the sediment surface (2–6 mm depth) where nitrification occurs can only give rise to low nitrate concentrations in the sediment surface layer, as seen from the measured and calculated nitrate profiles in Fig. 4. The best agreement between measured and calculated nitrate profiles at the Kattegat station were obtained using 5 mm as the depth of the nitrification zone, corresponding to the depth of oxygen penetration (10). If 5 mm is used as a mean depth of the nitrification zone in the sediment, a rate of 0–600 nmol N cm^{-3} d^{-1} in the surface 0.5 cm of the sediment is obtained. This is in good accordance with nitrification rates measured by Billen (2) in sediments from the southern Bight of the North Sea (0–480 nmol N cm^{-3} d^{-1} in the 0–1 cm layer). This agreement substantiates the conclusion that the method described gives near in situ nitrification rates.

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