Microzonation of Denitrification Activity in Stream Sediments as Studied with a Combined Oxygen and Nitrous Oxide Microsensor

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Microzonation of denitrification was studied in stream sediments by a combined O2 and N2O microsensor technique. O2 and N2O concentration profiles were recorded simultaneously in intact sediment cores in which C2H2 was added to inhibit N2O reduction in denitrification. The N2O profiles were used to obtain high-resolution profiles of denitrification activity and NO3− distribution in the sediments. O2 penetrated about 1 mm into the dark-incubated sediments, and denitrification was largely restricted to a thin anoxic layer immediately below that. With 115 μM NO3− in the water phase, denitrification was limited to a narrow zone from 0.7 to 1.4 mm in depth, and total activity was 34 nmol of N cm−2 h−1. With 1,250 μM NO3− in the water, the denitrification zone was extended to a layer from 0.9 to 4.8 mm in depth, and total activity increased to 124 nmol of N cm−2 h−1. Within most of the activity zone, denitrification was not dependent on the NO3− concentration and the apparent Ks for NO3− was less than 10 μM. Denitrification was the only NO3−-consuming process in the dark-incubated stream sediment. Even in the presence of C2H2, a significant N2O reduction (up to 30% of the total N2O production) occurred in the reduced, NO3−-free layers below the denitrification zone. This effect must be corrected for during use of the conventional C2H2 inhibition technique.

Recent development of in situ techniques has demonstrated the importance of denitrification in nitrogen cycling in both freshwater and marine sediments (8, 10, 11, 13, 14, 21, 33). Among the assays introduced for measuring in situ denitrification in undisturbed sediment cores, the acetylene inhibition technique appears to be the most convenient method (6, 24). By this method the C2H2, which inhibits the reduction of N2O to N2 in denitrifying bacteria (4, 41), is injected directly into the sediment and accumulation of N2O is taken as a measure of denitrification activity (34).

For a better understanding of the distribution and regulation of sediment denitrification, it is essential to develop new techniques by which the process can be studied at a higher spatial resolution. Recent attempts have been made to determine both NO3− concentration profiles (12, 15) and denitrification activity (19) by sectioning sediment cores in 1- to 5-mm-thick segments. The depth penetration of NO3− is sometimes limited to a very narrow zone, however, and sectioning in the soft and flocculent surface layer may be impossible.

In the study of O2 transformations in sediments, the O2 microelectrodes have proven to be extremely useful (28). From extension of this work, a new microsensor has recently been developed by which O2 and N2O concentrations in sediment, biofilm, etc., can be detected simultaneously on a microscale (30). After C2H2 is introduced, the development of N2O concentration profiles can be followed and denitrification can be quantified at a very high spatial resolution. In the present study, the microsensor was applied to study denitrification activity in lowland stream sediments. The distribution of activity was measured together with O2 and NO3− profiles on a microscale, and the results were compared with the activities that were determined by the conventional C2H2 inhibition technique.

MATERIALS AND METHODS

Study sites. The study was carried out in three Danish lowland streams, Århus Å, Gudena, and Døde Å, all of which are located in the eastern part of Jutland, Denmark. Sediment cores were sampled in 10-cm-long and 2.4-cm-wide Plexiglas tubes during summer (June 1987), when NO3− concentrations in the stream water were about 100 μM (Århus Å), 600 μM (Gudena), and 1,000 μM (Døde Å). The sediments were fine sand and silt, with an organic content of typically 3 to 5% (ignition loss) in the upper 2 cm. After sampling, the cores were stored overnight at room temperature under aerated stream water from the sampling locality.

O2 and N2O concentration profiles measured by use of a microsensor. To determine the microzonation of denitrification, a recently developed polarographic microsensor for simultaneous O2 and N2O measurements was used (30). In short, the sensor has both a gold-plated and a silver-plated cathode behind a gas-permeable membrane of silicone rubber at the sensor tip (diameter, 20 μm). By charging the cathodes against an Ag/AgCl anode (−1.04 and −1.18 V, respectively), the O2 diffusing into the sensor is reduced at the gold cathode, while N2O is reduced at the silver cathode. The silver cathode is situated slightly behind the gold cathode, and O2 therefore does not interfere with N2O measurements. The 90% response time was 1.5 s for O2 and 12 s for N2O, and the detection limit for both gases was about 1 μM.

Acetylene was added to the undisturbed sediment core by injecting C2H2-saturated distilled water through small silicocene-filled holes in the Plexiglas tube (10). Several injections of small portions ensured a homogeneous distribution of C2H2 in the upper 2 cm of the sediment, and a final concentration of approximately 10 kPa in the pore water was obtained. A total of 10% of the water phase in the cores was replaced by C2H2-saturated stream water, and the cores were incubated in the dark at 22°C. The overlying water was stirred by a small rotating magnet during the experiment.

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The microsensor was lowered into the sediment with a micromanipulator (Mertzheuser, Steinendorf/Wetzlar, Federal Republic of Germany), and changes in the O₂ and N₂O profiles could be followed over time at exactly the same point. After each determination of O₂ and N₂O profiles, the sensor was withdrawn from the sediment and calibrated in air-saturated water and in N₂O standard solutions.

**Denitrification and NO₃⁻ concentration profiles determined by modeling.** Apparent diffusion coefficients for N₂O and NO₃⁻ in the sediment were estimated from recorded coefficients of O₂, assuming a similar ratio between the values as between those in pure water (7, 26). The apparent diffusion coefficient of O₂ was determined in HgCl₂-inactivated sediment from Århus Å by using an O₂ microelectrode technique (27a). In the upper 1 mm of the sediment, the coefficient was 80% of the value in pure water, and the porosity (vol/vol) was 0.8. In layers below 1 mm, these values were 70% and 0.7, respectively.

The measured N₂O profiles were simulated by a diffusion-reaction model (29) to determine the depth distribution of N₂O production, i.e., denitrifying activity. The model was based on an extended version of Fick’s second law of diffusion (5):

\[ \frac{\partial C(x,t)}{\partial t} = D(x) \frac{\partial^2 C(x,t)}{\partial x^2} + \]

\[ \frac{\partial D(x)}{\partial x} \cdot \frac{\partial \phi(x)}{\partial x} + \frac{\partial C(x,t)}{\partial x} + [P(x) - R(x)]. \]  

For a given depth x, C(x,t) is the concentration at time t; \( D(x) \) is the apparent diffusion coefficient; \( \phi(x) \) is the porosity, and \( P(x) \) and \( R(x) \) are production and reduction rates, respectively. \( R(x) = 0 \) if N₂O reduction is completely inhibited by C₂H₂. Rates of N₂O production and reduction were inserted until the modeled concentration profiles closely simulated the measured ones.

Profiles of NO₃⁻ concentration could be obtained from the depth distribution of N₂O production rates under the assumptions that denitrification was the only NO₃⁻-consuming process and that NO₃⁻ production was 0 because of inhibition of nitrification by C₂H₂ (16, 39). The NO₃⁻ reduction rates were then twice the N₂O production rates on a molar basis, and the downward diffusion flux of NO₃⁻ at each depth \( [F(x)] \) could be calculated as the sum of NO₃⁻ consumption in all underlying layers. In accordance with Fick’s first law of diffusion:

\[ F(x) = \phi(x) \cdot D(x) \cdot \frac{\partial C}{\partial x}; \]

the change in NO₃⁻ concentration with depth \( (\partial C/\partial x) \) is then obtained from the flux divided by the apparent diffusion coefficient for NO₃⁻ and the porosity. All calculations were relatively simple since the depth variation of NO₃⁻ consumption, apparent diffusion coefficient, and porosity was described as constants for discrete depth intervals in the sediment. Assuming an NO₃⁻ concentration of 0 at the bottom of the N₂O production zone, the entire NO₃⁻ concentration profile was eventually constructed by a cumulation of the \( \partial C/\partial x \) values from the bottom of the denitrification zone to the water column. The NO₃⁻ profile thus gave a simulated NO₃⁻ concentration in the water phase which could be compared with the measured one as an independent test of the validity of the profile.

**Denitrification determined by the conventional C₂H₂ inhibition technique.** A separate experiment compared the determination of denitrification by the microsensor technique and by the conventional C₂H₂ inhibition technique with headspace extraction and gas chromatographic determination of N₂O (2, 10, 34). A set of five sediment cores with an overlying water column of approximately 4 cm in height was sampled in Århus Å. Acetylene was added to both the sediment and the water phase as described above, and gas-tight Plexiglas caps were mounted on top of the tubes. Small magnetic bars placed underneath the caps were moved by using a large external magnet. After 10 min, water samples (2 ml) were taken by syringe and frozen for later analysis of NO₃⁻ (initial concentration). The volumes removed were replaced by equivalent amounts of air, and the incubation was continued in darkness at 22°C. After 1.5 h of incubation, the heights (volumes) of the water and sediment phases were recorded. While the caps were kept on the tubes, water samples (2 ml) were taken for determination of NO₃⁻ (final concentration).

The N₂O accumulation in the water phase was determined in a 21-ml water sample which was injected into a closed, preevacuated, 40-ml serum flask. The flask was shaken vigorously for 2 min to obtain equilibrium, and a 1-ml gas sample was transferred to a preevacuated, 3-ml tube (Venject; Terumo Europe N.V., Leuven, Belgium) for later analysis of N₂O. In two of the five cores, N₂O concentration profiles were measured by use of the microsensor before the cores were sacrificed for the headspace extraction. The caps were removed from these cores immediately after the 21-ml water sample was taken, and the sensor was carefully lowered into the sediment by the micromanipulator. For both cores, a set of three concentration profiles was recorded before the tubes were capped again. The amount of N₂O that accumulated in the sediment plus the remaining fraction of the water phase was then determined in all five cores by the headspace extraction technique; the entire tube was shaken vigorously for 2 min before a 1-ml gas sample from the headspace (25 ml) was transferred to a Venject tube.

All NO₃⁻ concentrations were determined colorimetrically in an autoanalyzer (Chemlab Instruments Ltd., Essex, England) by the method of Armstrong et al. (3). Gas samples were analyzed for N₂O on a gas chromatograph (model 427; Packard Instrument Co., Inc., Rockville, Md.) equipped with a 60Ni electron capture detector (320°C). The components were separated on a 80/100 mesh Porapak Q column (length, 2 m; width, 3.2 mm) at a temperature of 60°C with pure N₂ used as the carrier gas (flow rate, 15 ml min⁻¹). In the headspace analysis, a correction was made for dissolved N₂O by using a Bunsen solubility coefficient of 0.65 (40). Total denitrification activity, expressed in nanomoles of nitrogen per square centimeter per hour, was estimated from the N₂O accumulation in the cores, i.e., the sum of N₂O in the water sample and in the suspension of sediment and water.

**RESULTS**

Depth distribution of denitrification activity. (i) Gudénä with 550 μM NO₃⁻ in the water. In an experiment with sediment from Gudénä and an NO₃⁻ concentration in the water phase of 550 μM, modeling of the N₂O concentration profiles showed that denitrification was distributed in a zone about 3.6 mm deep and starting immediately below the sediment surface (Fig. 1B). A good fit of the recorded N₂O concentration profiles was obtained throughout the incubation period (Fig. 1A). Within the denitrification zone, the
activity was almost constant and decreased only at the bottom. By accumulating all activities, a total denitrification rate of 99 nmol of N cm\(^{-2}\) h\(^{-1}\) was calculated.

The simulated NO\(_3^-\) profile gave an NO\(_3^-\) concentration of 556 \(\mu\)M in the water phase, which was in excellent agreement with the measured concentration of 550 \(\mu\)M (Fig. 1B).

(ii) Århus Å with 115 \(\mu\)M NO\(_3^-\) in the water. In C\(_2\)H\(_2\)-treated Århus Å sediment, O\(_2\) and N\(_2\)O concentration profiles were measured at the in situ NO\(_3^-\) concentration in the water phase (Fig. 2A). After 110 min, there was no further change in the N\(_2\)O profile, indicating a steady-state condition where N\(_2\)O production equaled N\(_2\)O diffusion loss plus consumption. This steady-state profile was stable for more than 2 h. Denitrification activity was estimated by modeling the steady state (equation 1), and the NO\(_3^-\) concentration profile was simulated; both are shown together with the measured O\(_2\) profile in Fig. 2B. Activity was limited to a narrow zone from 0.7 to 1.4 mm in depth in the sediment. Oxygen penetrated to a depth of about 1 mm, and denitrification activity was thus only found when O\(_2\) was either present at a very low concentration (<10 \(\mu\)M) or totally absent (Fig. 2B). A vertical integration of the activity gave an overall denitrification rate of 34 nmol of N cm\(^{-2}\) h\(^{-1}\).

The steady-state profile could be used to predict the time course of N\(_2\)O accumulation after C\(_2\)H\(_2\) addition. It was evident that shortly after C\(_2\)H\(_2\) addition (7 and 24 min of incubation; Fig. 2A), the measured N\(_2\)O concentrations in the sediment were considerably lower than those predicted by the model by using steady-state conditions.

The steady-state N\(_2\)O profile further revealed that even in the presence of C\(_2\)H\(_2\), a significant N\(_2\)O reduction took place in the deeper layers below the denitrification zone. The linearity of the N\(_2\)O profile below the production zone (from 1.4 to 3.5 mm in depth) indicated that the zone of N\(_2\)O reduction was separated in depth from the zone of production (Fig. 2A) and by simulation of the steady-state profile: a reduction rate of 16 nmol of N\(_2\)O-N cm\(^{-3}\) h\(^{-1}\) [\(R(x)\) value of equation 1] was obtained for sediments below 3.5 mm. An overall reduction rate of 10 nmol of N\(_2\)O-N cm\(^{-2}\) h\(^{-1}\), corresponding to 29\% of the production rate, was found.

The simulated NO\(_3^-\) profile gave an NO\(_3^-\) concentration of 117 \(\mu\)M in the water phase, which was identical to the measured concentration of 115 \(\mu\)M. In the denitrification zone, the availability of NO\(_3^-\) was lower because of the diffusion barrier of the oxic surface layer; the NO\(_3^-\) concentration at the upper edge of the denitrifying zone was about 30 \(\mu\)M (Fig. 2B).

(iii) Århus Å with 1,250 \(\mu\)M NO\(_3^-\) in the water. In a separate core from Århus Å, the water column was amended with NO\(_3^-\) to a final concentration of 1,250 \(\mu\)M, which was a 10-fold increase compared with in situ conditions. The core was preincubated for 5 h at room temperature before the C\(_2\)H\(_2\) was added and the O\(_2\) and N\(_2\)O profiles were recorded.

Compared with the sediment with in situ NO\(_3^-\) concentrations (Fig. 2), the zone of denitrification activity was markedly deeper in the NO\(_3^-\)-amended sediment; denitrification was thus observed from 0.9 to 4.8 mm below the sediment surface (Fig. 3B). Also in this sediment, the activity was relatively constant throughout most of the denitrification zone, and the rates (per unit volume of sediment) were not significantly different from those obtained at the in situ NO\(_3^-\) concentration. Accumulation of the whole activity profile gave an overall denitrification rate of 124 nmol of N cm\(^{-2}\) h\(^{-1}\), which was 3.5-fold higher than that at the in situ NO\(_3^-\) concentration.

No discrepancy between measured and simulated N\(_2\)O concentration profiles was observed in the early phase of the
incubation with C$_2$H$_2$ (Fig. 3A), but the simulations revealed a significant N$_2$O reduction in the deeper part of the sediment. Thus, for sediment below 5 mm in depth, a reduction rate of 22 nmol of N cm$^{-3}$ h$^{-1}$ was used in the model [$R(x)$ value of equation 1] to give a perfect simulation of the N$_2$O concentration profiles. Steady-state conditions were not obtained during the incubation period of 55 min; until a steady state was reached, the overall N$_2$O reduction increased along with the extension in depth of the N$_2$O profile. An overall reduction rate of 8 nmol of N$_2$O-N cm$^{-2}$ h$^{-1}$, corresponding to 7% of the total N$_2$O production, was calculated for 55 min of incubation.

The simulated N$_2$O profile gave a concentration of 845 µM at the upper edge of the denitrification zone and a concentration of 1,170 µM in the overlying water phase (Fig. 3B); the latter was very close to the measured concentration of 1,250 µM.

Comparison of denitrification activity measured by the microsensor and by the conventional C$_2$H$_2$ inhibition techniques. To compare determination of N$_2$O accumulation by the microsensor and by the conventional C$_2$H$_2$ inhibition techniques (headspace extraction followed by gas chromatographic detection of N$_2$O), both techniques were applied to sediment cores from Århus Å with the in situ N$_2$O concentration of 110 µM in the overlying water. By the conventional technique, N$_2$O accumulation was determined by headspace extraction in five parallel cores after 1.5 h of incubation with C$_2$H$_2$. Since nitrification was inhibited by C$_2$H$_2$, total N$_2$O consumption could be estimated from the decreasing N$_2$O$^-$ concentration in the water phase during the denitrification assay. Mean activity (± standard error of the mean) for total N$_2$O$^-$ consumption and denitrification in the five cores were 62 ± 2 and 53 ± 3 nmol of N cm$^{-2}$ h$^{-1}$, respectively, and N$_2$O accumulation thus accounted for 85% of the total N$_2$O$^-$ consumption.

Nitrous oxide concentration profiles were measured by the microsensor technique in two of the five cores just before the headspace extraction (Fig. 4A and B). The profiles gave an indication of the heterogeneity in spatial distribution of the denitrification. While one of the cores from Århus Å only showed little variation in depth distribution and total activity (Fig. 4A), the other one had N$_2$O maxima at different depths and showed a significant variation in the recorded accumulation of N$_2$O (Fig. 4B). On the basis of six N$_2$O concentration profiles measured in two cores, the accumulated N$_2$O content made up only 64% of the N$_2$O content measured by the conventional C$_2$H$_2$ inhibition technique. For comparison, N$_2$O concentration profiles were also measured in a C$_2$H$_2$-treated sediment core from Døde Å (after 1.5 h of incubation) with an N$_2$O$^-$ concentration of 1,000 µM in the water phase (Fig. 4C). At this locality, the sediment surface was visually heterogeneous because of faunal activity, and the recorded N$_2$O profiles were highly variable within the same sediment core. Thus, one of the profiles showed a very irregular shape, with no distinct maximum of N$_2$O accumulation, and for the three recorded profiles, a threefold or greater variation was found for the total amount of N$_2$O accumulated.

**DISCUSSION**

Depth distribution of denitrification activity. The combined O$_2$ and N$_2$O microelectrode has made it possible for the first time to demonstrate and study the microzonation of denitrification in sediments. In the lowland streams investigated here, we localized denitrification activity in a narrow zone.
within the upper few millimeters of the sediment and demonstrated that the activity zone was primarily determined by the depth penetrations of O_2 and NO_3^-.

(i) Denitrification activity in relation to O_2. Denitrification at low O_2 tension has previously been described for both marine sediments and soils (18, 38), but truly aerobic denitrification such as that described for a denitrifying mixotroph (32) was never observed in the investigated sediments. The results indicated that there was a small amount of activity just above the oxic/anoxic interface (O_2 concentrations from 0 to 20 \mu M), but the major fraction of the denitrification was found in completely anoxic layers. The oxic surface layer therefore constitutes a barrier for NO_3^- diffusion from the water phase to the denitrification zone, and the thickness of

FIG. 3. (A) Measured (datum points) and simulated (solid lines) N_2O profiles after various incubation periods following C_2H_2 addition to a sediment core from Århus Å with 1,250 \mu M NO_3^- in the water phase. (B) Depth distribution of denitrification activity obtained by modeling of the time course of N_2O accumulation. Measured O_2 and simulated NO_3^- profiles are indicated.

FIG. 4. N_2O concentration profiles in sediment from Århus Å (A and B) and Døde Å (C). The three lines in each panel represent different N_2O profiles obtained in the same core. The NO_3^- concentrations of the water phase were 115 \mu M (A and B) and 1,000 \mu M (C). All profiles were recorded after 1.5 h of incubation with C_2H_2. Note the change of scale in panel C.
the barrier may be a major controlling factor for denitrification in stream sediments (L. P. Nielsen, P. B. Christensen, N. P. Revsbech, and J. Sørensen, manuscript in preparation).

(ii) Denitrification activity in relation to NO$_3^-$ concentration. All simulations of NO$_3^-$ profiles gave NO$_3^-$ concentrations in the water phase that were almost identical to those actually measured, indicating that denitrification indeed was the only NO$_3^-$-consuming process in the sediment. The close agreement between measured and simulated NO$_3^-$ concentrations also indicated that the apparent diffusion coefficient for NO$_3^-$ in the sediment was correctly estimated, assuming a ratio between diffusion coefficients of O$_2$, N$_2$O, and NO$_3^-$ in the sediment similar to that in pure water. A specific reduction of NO$_3^-$ diffusion because of electrical interactions between the NO$_3^-$ ion and charged surfaces, as observed in trickling filter matrices (31), was apparently unimportant in the sediments since the estimated NO$_3^-$ profile otherwise would have underestimated the NO$_3^-$ concentration of the water phase. We therefore concluded that the estimated NO$_3^-$ profiles described the real NO$_3^-$ concentration profiles in the sediments.

Denitrification was limited to a narrow zone of 0.7 mm in depth in the Århus A sediment when NO$_3^-$ was present at a relatively low concentration (110 µM) in the water phase (Fig. 2B). At a higher NO$_3^-$ concentration (1,250 µM), the denitrification zone was extended to a 4-mm-deep zone (Fig. 3A). The activities recorded in the denitrification zone were not stimulated by higher NO$_3^-$ availability per se. Thus, even if the NO$_3^-$ concentration was increased from 30 to 850 µM at the upper edge of the activity zone in the Århus A sediment, the specific activities within the zone were still comparable (Fig. 2B and 3B). Furthermore, the activity seemed to be constant throughout the denitrification zone in the sediments from both Gudøen (Fig. 1B) and Århus A (Fig. 3B). Only at the lower edge of the activity zone, where NO$_3^-$ dropped to a concentration below 10 µM, the activity decreased significantly. This indicated a low apparent K$_m$ value (<10 µM) for the reduction of NO$_3^-$ by denitrification in these sediments.

The depth distribution of denitrification activity has previously been illustrated by vertical sectioning of C$_2$H$_4$-incubated sediment cores (2, 11, 19). From data obtained with the microsensor, it was possible to define the spatial microzonation of denitrification accurately and in much greater detail than before. Denitrification was never recorded at depths below 5 mm in the sediment, and several earlier observations of denitrification activity below a 1-cm depth in such sediments may therefore be erroneous because of the downward diffusion of N$_2$O during incubation. Exceptionally deep zones of denitrification can only be found where bioturbation by sediment fauna, macrophyte root activity, or upwelling of NO$_3^-$-rich groundwater provides an additional NO$_3^-$ source in the deeper layers (1, 10, 11).

Combination of the microsensor and conventional C$_2$H$_4$ inhibition techniques. Both the conventional C$_2$H$_4$ inhibition technique and the microsensor technique have advantages and disadvantages, when used alone. The former technique may be preferable when determining an overall activity in sediments, while the latter should be used when detailed studies of the denitrification process are required. However, by combining the two techniques, even more information on the denitrification can be obtained and a better estimate of the activity can be given.

(i) Denitrification in relation to sediment heterogeneity. By using the microsensor, an in situ activity of 34 nmol of N cm$^{-2}$ h$^{-1}$ was obtained in the Århus A sediment (Fig. 1). This activity was significantly lower than both the NO$_3^-$ consumption rates (62 nmol of N cm$^{-2}$ h$^{-1}$) and the N$_2$O accumulation rates (53 nmol of N cm$^{-2}$ h$^{-1}$) obtained by the conventional C$_2$H$_4$ technique. When the microsensor was applied, however, activity was recorded within a very small volume of sediment, and so several microprofiles must be measured to compensate for heterogeneity (Fig. 4A and B). The variability of N$_2$O profiles can probably be explained by the presence of faunal burrows that create microsites with a higher activity than that in the bulk sediment (1, 9, 25). Such a faunal activity was confirmed from visual inspection of the cores from Døde A and seemed to be responsible for the very heterogeneous distribution of denitrification in this sediment (Fig. 4C).

(ii) Disturbance of in situ NO$_3^-$ profile by C$_2$H$_4$ injection. A distortion of activity caused by the injection of C$_2$H$_4$ was observed in the Århus A sediment (110 µM NO$_3^-$ in the water phase). Marked disagreement between measured and simulated N$_2$O profiles was demonstrated in the early phase of incubation; the two first N$_2$O profiles recorded at 7 and 24 min after C$_2$H$_4$ addition were located distinctly higher in the core than the following ones, and the accumulated amount of N$_2$O was significantly lower than expected (Fig. 2A). When C$_2$H$_4$-saturated, distilled water was injected into the sediment, the pore water NO$_3^-$ profile, which only extended about 1.5 mm into the sediment (Fig. 2B), was apparently displaced upwards and denitrification activity was impeded because of lower NO$_3^-$ availability until a steady-state NO$_3^-$ profile was reestablished. The original NO$_3^-$ profile was eventually recovered after 50 min of incubation (data not shown). This distortion is most pronounced in sediments in which denitrification activity is located immediately below the sediment-water interface. In such cases, the addition of C$_2$H$_4$ to the water phase alone may be the preferred choice, since rapid diffusion of C$_2$H$_4$ to the denitrification zone should allow measurements of N$_2$O accumulation from undisturbed concentration gradients. By comparison, the injection of C$_2$H$_4$-saturated water into Gudøen sediment and N$_2$O-enriched Århus A sediment did not cause any significant displacement of the N$_2$O profiles since NO$_3^-$ here extended to a depth of about 5 mm (Fig. 1B and 3B).

(iii) Incomplete inhibition of N$_2$O reduction by C$_2$H$_4$. By the microsensor technique, it was also possible to demonstrate a significant N$_2$O reduction in the C$_2$H$_4$-treated sediment. In the Århus A sediment with an in situ NO$_3^-$ concentration in the water phase, N$_2$O reduction made up about 30% of the total N$_2$O production under steady-state conditions; a similar value has been observed in other stream sediments (Nielsen et al., in preparation). There have been several reports on low efficacy of the C$_2$H$_4$ inhibition at low NO$_3^-$ concentrations (20, 22, 27). In the sediments used in this study, however, the close agreement between the predicted and the measured NO$_3^-$ concentrations in the water phase excluded the possibility that significant N$_2$O reduction could take place within the production (denitrification) zone. Also, the N$_2$O profiles demonstrated that the production and reduction zones of N$_2$O were separated in depth (Fig. 2A), and we concluded that N$_2$O reduction occurred only in the deeper, NO$_3^-$-free layers. The incomplete blockage of N$_2$O reduction was therefore most likely caused by the presence of sulfide, which alleviates the C$_2$H$_4$ inhibition even at low concentrations (36, 37).

The N$_2$O reduction in the presence of C$_2$H$_4$ represents a source of error in the estimate of total denitrification by the conventional C$_2$H$_4$ inhibition technique. However, the N$_2$O
consumption can be quantified by the microsensor technique and a correction factor can be determined. Because of the $N_2O$ reduction in the presence of $C_2H_2$, a difference between $NO_3^-$ consumption and $N_2O$ accumulation may not directly indicate or quantify an alternative pathway of $NO_3^-$ reduction. In the Århus Å sediment, for instance, the lower rates of $N_2O$ accumulation relative to the total $NO_3^-$ consumption rate must thus be explained by the occurrence of $N_2O$ reduction in the presence of $C_2H_2$: rates of $NO_3^-$ consumption in this study were a better estimate of total denitrification activity than the $N_2O$ accumulation rates were. As discussed above, the simulations of $NO_3^-$ profiles also indicated that denitrification is the only $NO_3^-$-consuming process in the sediments studied here. The nitrate ammonification (dissimilatory $NO_3^-$ reduction to $NH_4^+$) described in marine sediments (17, 23, 24, 35) therefore did not seem to be important in the streams that we investigated.

In conclusion, the combined $O_2$-$N_2O$ microsensor was valuable in the study of microzonation of the denitrification process, and its use in combination with the conventional $C_2H_2$ inhibition technique and flux measurements may greatly improve the study of denitrification and other $NO_3^-$-consuming processes in sediments.

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LITERATURE CITED


