

## Microelectrode Measurements of Nitrate Gradients in the Littoral and Profundal Sediments of a Meso-Eutrophic Lake (Lake Vechten, The Netherlands)

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**NO<sub>3</sub><sup>-</sup> concentration profiles were measured in the sediments of a meso-eutrophic lake with a newly developed microelectrode. The depth of penetration of NO<sub>3</sub><sup>-</sup> varied from only 1.3 mm in organic-rich profundal silty sediments to 5 mm in organic-poor littoral sandy sediments. The thickness of the zone of denitrification in the organic-rich sediments was <500 μm. Oxygen profiles measured simultaneously revealed that the zone of denitrification was directly adjacent to the aerobic zone. The results demonstrate high denitrification rates (0.26 to 1.31 mmol m<sup>-2</sup> day<sup>-1</sup>) at in situ nitrate concentrations in the overlying water (0.030 mmol liter<sup>-1</sup>) and limitation of denitrification by nitrate availability.**

Nitrate is an important intermediate in the biological nitrogen cycle and serves as the electron acceptor for dissimilatory nitrate reduction and denitrification, as a nitrogen source for cell growth (assimilatory nitrate reduction), and as an end product of nitrification. The distribution of nitrate in sediment pore water is controlled by concentrations in the overlying water, the rate of transport into the sediments, and the bacterial production and consumption in the sediments. Detailed information about activity sites and rates of conversion can be obtained by fine-scale nitrate concentration profiles. Nitrate concentration profiles have been conventionally obtained by measuring nitrate in the extracted pore water from subsamples of sediment cores (2) and in the pore water of in situ membrane samplers (1). In all previous measurements, the spatial resolution was limited to a range of several millimeters to a few centimeters. Consequently, accurate nitrate concentration profiles could not previously be determined in undisturbed environments where the conversions and subsequent gradients occurred in a relatively small zone. The most direct way of measuring solutes without disturbance of the physical environment is by means of microelectrodes. In fact, the introduction of oxygen microelectrodes in aquatic ecology has significantly improved the understanding of microbial processes at aquatic interfaces (6, 8, 13). Recently, a microelectrode for measuring ammonium was developed (D. De Beer and J. C. Van den Heuvel, Talanta, in press). A new microelectrode which offers the same high spatial resolution has now been developed for NO<sub>3</sub><sup>-</sup>.

This study reports the first use of this liquid membrane nitrate microelectrode. Nitrate concentration profiles were measured in the two dominant sediment types of a small meso-eutrophic lake: sandy organic-poor littoral sediments and silty organic-rich profundal sediments. The nitrate microelectrode made it possible to measure fine-scale nitrate profiles at the sediment-water interface at natural concentrations and enabled the determination of the sites of activity. Nitrate conversion rates in the sediments were calculated from the concentration gradients of nitrate and from measured diffusion coefficients.

**Nitrate microelectrode and measuring circuit.** Nitrate microelectrodes consisted of 1.2-mm-diameter glass capillaries with a length of 12 cm and an external tip diameter of 1 μm. The tips of the electrodes were silanized with a 20% (vol/vol) solution of trimethylchlorosilane in carbon tetrachloride and filled with sensor. The liquid membrane used was a model 92-07-01 nitrate exchanger for macroelectrodes (Orion Research Inc., Cambridge, Mass.). The electrode shafts were filled with electrolyte solution (40 mM KNO<sub>3</sub>). The construction of the microelectrode is described elsewhere (D. de Beer and J.-P. R. A. Sweerts, *Anal. Chim. Acta*, in press).

The measuring circuit during calibration consisted of Ag/AgCl, 0.3 M KCl, 40 mM KNO<sub>3</sub>, ion-selective membrane, sample solution, 3 M KCl agar bridge, 0.3 M KCl, AgCl/Ag. The electrode assembly was placed in a Faraday cage. Calibration was done in a continuous-flow cell. The solutions inside and outside the Faraday cage were electrically separated by drip chambers. The electrical potential was conducted through a triaxial input cable (Keithley 6011) to an electrometer (Keithly 617) placed outside the Faraday cage. The response of the nitrate microelectrodes to concentrations of 10<sup>-1</sup> to 10<sup>-5</sup> M KNO<sub>3</sub> was linear, with a slope of 55 mV log [KNO<sub>3</sub>], and concentrations were measured accurately to 10<sup>-6</sup> M. The response time was about 30 s, and the drift of the signal was about 3 mV h<sup>-1</sup>. Selectivity factors were determined by using the separate-solution method. The selectivity factors for Cl<sup>-</sup> and NO<sub>2</sub><sup>-</sup> were 0.006 and 0.06, respectively, of the same order as those specified by Orion for macroelectrodes (0.004 and 0.04, respectively). Therefore, selectivity factors for other ions were expected to be almost the same as those specified by Orion. Caution should be taken in media with relatively high concentrations of interfering ions. As an example, the electrodes are not applicable in high-salt marine environments because of the sensitivity for Cl<sup>-</sup> (0.006). The electrodes were stable for at least 5 h. For each experiment freshly prepared electrodes were used. The electrode signal was not influenced by the velocity of the liquid.

**Oxygen concentration profiles.** Oxygen profiles were measured with a membrane-covered cathode-type microelectrode with a tip diameter of 7 μm (Delta Institute, Yerseke, The Netherlands) and an external reference electrode (7). At

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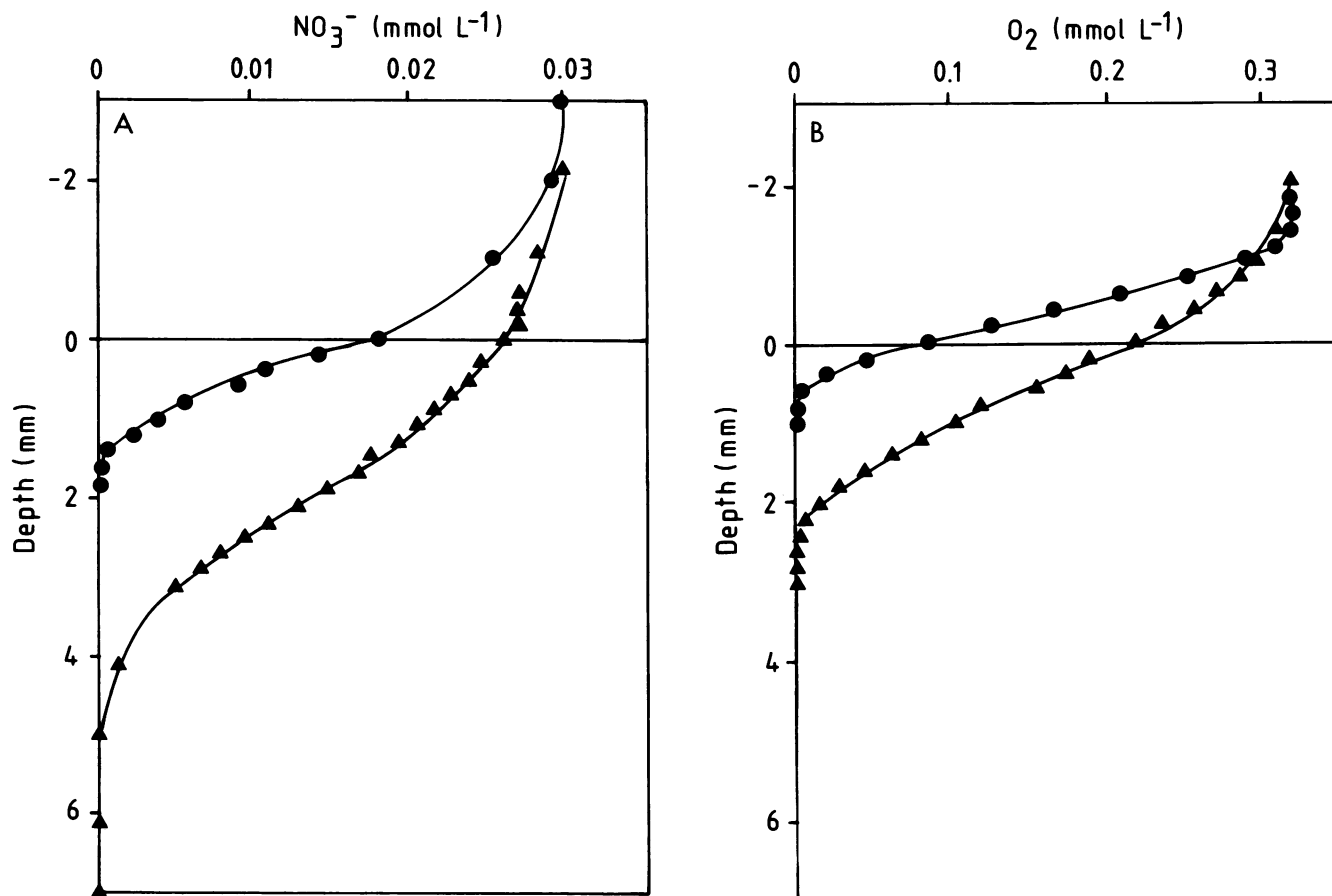


FIG. 1. Nitrate and oxygen profiles in the sediments of Lake Veichten. (A) Nitrate profiles in and above sand (▲) and silt (●). (B) Oxygen profiles in and above sand (▲) and silt (●).

100% air saturation, the oxygen microelectrode output of this particular electrode increased by 2% at a water velocity of  $1 \text{ cm s}^{-1}$  as compared with a stagnant-water column. Positioning of the microelectrodes was accomplished by a micromanipulator (resolution,  $5 \mu\text{m}$ ) (12).

**Sample sites and laboratory conditions.** Intact littoral (2-m depth) and profundal (10-m depth) sediment cores (diameter, 7 cm) were collected in December 1987, during the circulation period, from Lake Veichten. Lake Veichten is a small monomictic meso-eutrophic lake located in The Netherlands (11). The cores were stored at the in situ temperature ( $7^\circ\text{C}$ ) in the dark. All analyses were completed 12 to 24 h after sampling, unless otherwise indicated. During analysis, overlying water was stirred with an impeller at a rate sufficient to turbulently mix it and maintain the diffusive boundary layer at a thickness of 0.8 to 1.2 mm, as measured with oxygen microelectrodes (J.-P. R. A. Sweerts, V. St. Louis, and T. E. Cappenberg, *Freshwater Biol.*, in press).

**Flux calculations.** Nitrate fluxes at the sediment-water interface were calculated from the nitrate concentration profiles with Fick's first law of diffusion:

$$J \text{ NO}_3^- = \frac{D_0 \phi}{\theta^2} \cdot \frac{d\text{NO}_3^-}{dx}$$

where  $J \text{ NO}_3^-$  is the flux of nitrate over the sediment-water interface,  $D_0$  is the molecular diffusion coefficient for nitrate ( $1.22 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  at  $7^\circ\text{C}$ ) (3),  $\phi$  is the porosity at the

depth of calculation,  $\theta^2$  is the square of the tortuosity at the depth of calculation, and  $d\text{NO}_3^-/dx$  is the initial (linear) decrease in nitrate concentration in the sediments. The tortuosity squared of the sediments was calculated from measured diffusion coefficients for tritiated water in sediment pore water by using the  $^3\text{H}_2\text{O}$  diffusion technique (10). Porosity at the sediment-water interface was extrapolated from regression of the porosities of 5-mm sections with depth (10).

**Sediment analysis.** The organic carbon in the dried ( $60^\circ\text{C}$ ) and homogenized material was determined with a CHN analyzer (Carlo Erba Instrumentazione elemental analyzer, model 1106).

The nitrate profiles measured with a microelectrode in the sediments of a meso-eutrophic lake are presented in Fig. 1A. Nitrate concentrations in freshwater lakes are in general in the range of 0 to  $50 \mu\text{M}$ . The nitrate microelectrode measures accurately to  $10^{-6} \text{ M NO}_3^-$  and is therefore particularly suitable for measuring concentration gradients at the sediment-water interface in lakes. The nitrate profiles in each investigated sediment type were highly reproducible. The depth of penetration varied from 5 mm in the sandy organic-poor (organic C, 0.9% of dry weight) littoral sediments to only 1.3 mm in the silty organic-rich (organic C, 7.8% of dry weight) profundal sediments. The linear slope at the beginning of all the nitrate gradients indicated only diffusion in that part of the gradient, while both diffusion and

TABLE 1. Characteristics and calculated flux rates of nitrate microprofiles in lake sediments

Location	Depth (m)	Organic C (% of dry weight)	NO <sub>3</sub> <sup>-</sup> (mmol liter <sup>-1</sup> )	φ	θ <sup>2</sup>	$dNO_3^-/dx$ (mmol liter <sup>-1</sup> mm <sup>-1</sup> )	$J NO_3^-$ (mmol m <sup>-2</sup> day <sup>-1</sup> )
Sandy	2	0.9	0.030 <sup>a</sup>	0.57	1.4	0.006	0.26
Silty	10	7.8	0.030 <sup>a</sup>	0.93	1.2	0.016	1.31
Silty	10	7.8	0.102 <sup>b</sup>	0.93	1.2	0.052	4.25

<sup>a</sup> Natural nitrate concentration.

<sup>b</sup> Experimentally increased nitrate concentration.

consumption occurred in the deeper portion of the gradient (Fig. 1A).

Oxygen profiles were measured simultaneously with nitrate profiles (Fig. 1B). The oxygen gradients in the diffusive boundary layer had a linear slope as a result of diffusion only. Recently, aerobic denitrification and microaerophilic denitrification have been demonstrated in pure cultures of several denitrifying bacteria (9). Furthermore, the capacity to denitrify for several hours under aerobic conditions has been demonstrated for anaerobically cultured denitrifying bacteria isolated from freshwater and marine sediments (4). However, our data revealed that in both the silty and sandy sediments the zones of oxygen and nitrate consumption were separated and that nitrate consumption occurred in a narrow zone directly adjacent to the aerobic zone. In the silty sediments this zone was only approximately 500 μm in thickness. In the last decade it has been demonstrated, with

oxygen microelectrodes, that all oxygen diffusing into sediments can be consumed in a narrow zone in organic-rich sediments of lakes and oceans (8, 13). The results of this study demonstrate that anaerobic mineralization by denitrification can also occur in a narrow layer adjacent to the site of activity of aerobic mineralization.

Table 1 lists the approximate magnitudes of nitrate uptake rates calculated from the nitrate concentration profiles with Fick's first law of diffusion. The nitrate consumption rates in the silty sediments calculated in this study were four to seven times higher than the nitrate consumption rates as calculated from measurements of nitrate in the extracted pore water from subsamples of sediment cores (2). Nitrate consumption rates were of the same order as the results from <sup>15</sup>NO<sub>3</sub><sup>-</sup> dilution experiments with sediments from other lakes (10). However, with the <sup>15</sup>NO<sub>3</sub><sup>-</sup> dilution technique the production of N<sub>2</sub> is measured, and nitrate consumption rates are underestimated if other pathways and nitrogen end products are involved. Nitrate microelectrode measurements provide nitrate flux rates over the sediment-water interface without sediment treatments or disturbance.

The demonstrated high denitrification rates led to the question of whether denitrification is limited by the availability of nitrate. The nitrate concentration in the overlying water of silty sediments was therefore experimentally increased three to four times. Overlying water was agitated as previously described, and oxygen gradients did not change during the course of the experiment. After 2 days of equilibration, the nitrate consumption rates had increased greatly in silty sediments (Table 1). The penetration depth of nitrate increased only slightly (Fig. 2), and it can be concluded that nitrate consumption in the sediments was limited by its availability. In the last few decades nitrate levels in freshwater and marine systems have increased because of human activities (5, 10). The ultimate removal of nitrate as molecular nitrogen from aquatic systems is accomplished by denitrification in sediments. Also, denitrification increases alkalinity and contributes to the acid-neutralizing capacity of freshwater systems (10). The nitrate addition experiment demonstrated the high nitrate consumption capacity (Table 1) of the silty organic-rich sediments at nitrate levels that are the same as those in many acidified lakes in the north temperate zone (10).

The nitrate microelectrode made it possible to measure fine-scale nitrate profiles at the sediment-water interface of lake at natural concentrations. The measurements enabled accurate flux calculations and the determination of the site of activity.

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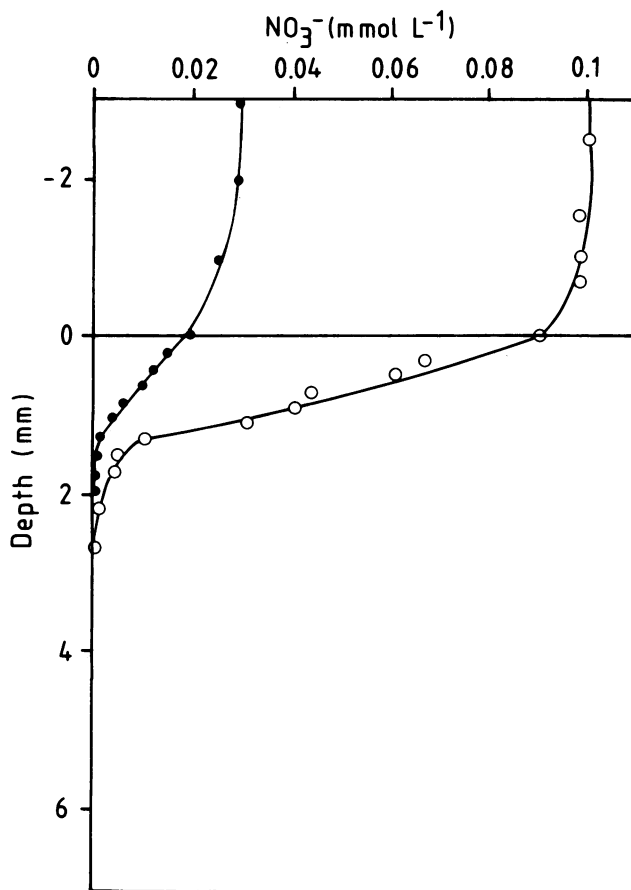


FIG. 2. Nitrate profiles in the silty sediments of Lake Vechten at natural (●) and experimentally increased (○) nitrate concentrations.

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