Denitrification in a coastal sediment measured in situ by the nitrogen isotope pairing technique applied to a benthic flux chamber

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ABSTRACT: A free operating benthic flux chamber lander (ELINOR) was used to measure in situ denitrification rates in the Aarhus Bight, Denmark (16 m depth). After insertion of the flux chamber into the sediment \(^{15}\)N-NO\(_3^-\) was automatically injected into the enclosed water phase. After 3 to 4 h of incubation ELINOR was brought back to the surface with an intact water and sediment phase. Dinitrogen was extracted and later analyzed for \(^{15}\)N enrichment by mass spectrometry. Parallel sediment cores were sampled for laboratory incubation under in situ conditions. In situ denitrification rates in June and November were 350 and 470 \(\mu\)mol N m\(^{-2}\) d\(^{-1}\) respectively, and were not significantly different from the laboratory measured rates of 290 and 500 \(\mu\)mol N m\(^{-2}\) d\(^{-1}\), respectively. Oxygen uptake was 40% lower in the laboratory incubated cores compared to in situ measurements, apparently due to lowered activity of the dominating species of infauna, the bivalve Abra alba. Nitrate concentrations were low in the bottom water (<8 \(\mu\)M) and most of the denitrification activity (65 to 100%) was coupled to nitrification in the sediment. A laboratory test showed that the addition of \(^{15}\)N-NO\(_3^-\) did not significantly impede the reduction rate of the natural unlabelled NO\(_3^-\). The results support the validity of laboratory measurements for coastal waters and demonstrate the potential of combining benthic flux chamber landers and the isotope pairing technique for accurate measurements of denitrification in shelf, deep sea, and freshwater environments.

KEY WORDS: Denitrification - Sediment - Benthic lander - Flux - \(^{15}\)N - Nitrogen - Nitrification

INTRODUCTION

Primary production in nearshore waters is largely controlled by the availability of combined nitrogen in the photic zone (Ryther & Dunstan 1971, Paasche 1988), and the cycling of nitrogen is therefore of prime interest in marine ecology. Denitrification in sediments is the major nitrogen sink in the sea and many attempts to quantify this process have been made (for review see Seitzinger 1988). The most widespread methods are based on laboratory incubations of sediment cores measuring either N\(_2\) efflux (Seitzinger et al. 1980), N\(_2\)O accumulation after acetylene inhibition of N\(_2\)O reduction (Sørensen 1978), \(^{15}\)N\(_2\) efflux after addition of \(^{15}\)N tracers (Nishio et al. 1983) or differences of NO\(_3^-\) fluxes and nitrification rates (Blackburn & Henriksen 1993). The methods were most recently reviewed by Seitzinger (1990), Koike (1990) and Revsbech & Sørensen (1990). Also, porewater profiles of nitrogen species have been used for estimating denitrification (Vanderbrughe et al. 1977).

In situ efflux of N\(_2\) from the sea bottom was recently measured using a benthic flux chamber lander (Devol 1991). A long incubation time was needed to detect increase of N\(_2\) concentration above the high background level, and oxygen in the chamber was therefore seriously depleted (Archer & Devol 1992), which probably affected denitrification rates by changing nitrification activity and diffusion of NO\(_3^-\) through the
oxic zone (Nielsen 1992, Rysgaard et al. 1994). The aim of the present study was to try the new nitrogen isotope pairing technique (Nielsen 1992) in situ and compare the denitrification rates obtained with those obtained by parallel laboratory measurements. The nitrogen isotope pairing technique has generally been shown to be sensitive and robust (Nielsen 1992, 1993, Rysgaard et al. 1993, 1995, Pelegri et al. 1994, Rysgaard-Petersen et al. 1994) and seems to eliminate important shortcomings of traditional core incubation methods (Seitzinger et al. 1993). A known amount of $^{15}$NO$_3^-$ is added to water overlying the sediment and the indigenous denitrification of unlabelled NO$_3^-$ is calculated from the formation of single labelled ($^{14}$N$^{15}$N) versus double labelled ($^{15}$N$^{15}$N) dinitrogen. The method assumes that addition of $^{15}$NO$_3^-$ does not influence denitrification of the natural NO$_3^-$ and that the isotopes are uniformly mixed in the denitrification zone (Nielsen 1992). These assumptions were also tested in this study.

**MATERIALS AND METHODS**

**Study site.** The study site at 16 m depth (Stn 6, 56°09'10" N, 10°19'20" E) in the Aarhus Bight, Denmark, has recently been the object of several integrated studies of carbon, nutrient and mineral cycling (K. Richardson & B. B. Jørgensen unpubl.). The sediment is silty with an organic matter content of 9 to 10% (dw) and a porosity of 0.83 (v/v) for the upper 6 cm. In situ measurements and simultaneous collection of cores for laboratory measurements were done on June 10 and November 24, 1992. An extra set of cores for testing of $^{15}$NO$_3^-$ concentration effects was collected on June 19.

**In situ procedure.** In situ measurements were made with the benthic flux chamber lander 'ELINOR' (Glud et al. 1993, 1995) modified from the BECI-lander (Jahnke & Christiansen 1989). After release the lander sank to the bottom and the respiration chamber (30 cm × 30 cm) was forced into the sediment. In this shallow water study the lander remained connected to a buoy at the surface. Optionally the lander can be equipped with buoyancy and automatically discharged ballast for free operation at deeper sites (Glud et al. 1994). Further operations were controlled by a preprogrammed computer unit of the lander. Closure of the chamber lid was triggered by burnwires after 1 h and the water phase was continuously mixed by a magnetic stirrer attached to the lid (9 r.p.m.). This created a diffusive boundary layer thickness of approximately 400 μm (Glud et al. 1995). The enclosed water column was 9 to 13 cm high. Oxygen concentrations were monitored continuously by two O$_2$ minisensors (Glud et al. 1995) mounted in the lid. About 10 min after closure 30 ml of 15 mM $^{15}$NO$_3^-$ in seawater was injected into the waterphase (final conc. 35 and 50 μM) from a spring driven 60 ml plastic syringe activated by a magnetic switch. During the incubation, water samples of 45 ml were withdrawn at predefined time intervals. The samples were stored in gastight ampoules for Winkler calibration of the electrodes. The O$_2$ concentration of the first samples which were stored in situ during the incubation equaled the bottom water value determined from water samples taken by a Niskin bottle. This indicated that the samples were taken without contamination and that the O$_2$ consumption in the samples could be ignored. Incubation time was up to 3 h, which ensured that O$_2$ was depleted by a maximum of only 25%. At the end of the incubation a burnwire triggered a hydraulic scoop which caught the undisturbed water and sediment phase (approx. 25 cm deep). ELINOR was withdrawn from the bottom and within 10 min brought on deck where 20 ml of a 50% (w/v) ZnCl solution was added to the water phase to stop biological activity. A laboratory test showed that O$_2$ penetration in the sediment started to increase within seconds after Zn addition, indicating an instant blockage of respiration processes (data not shown). In case the recovery of the lander is expected to take longer, ZnCl may also be injected at the bottom using an extra automatic syringe. The lid was opened, the water height measured and water samples for determination of $^{15}$N$_2$ accumulation were gently transferred by syringes to 12.4 ml glass vials containing 250 μl of the ZnCl solution. The vials were closed with gastight screw caps with injection septa (Exetainer, Labco) without entrapping any air bubbles. Samples for NO$_3^-$ analysis were taken in plastic vials. The water phase was always clear as were the recovered time series water samples indicating no significant disturbance of the sediment surface during incubation and recovery of the lander. Eight plexiglass cylinders (inner diameter, i.d. = 5 cm) were evenly inserted in the sediment and core samples with 6 to 9 cm sediment and 5 to 9 cm water were taken. To extract $^{15}$N$_2$ accumulated in the porewater, sediment and water in each core was gently mixed into a slurry from which samples were stored in gastight ampoules for determination of the isotopes are uniformly mixed in the denitrification zone (Nielsen 1992). These assumptions were also tested in this study.

**Laboratory incubations.** Sets of 8 sediment cores (i.d. = 5 cm) with 5 to 9 cm sediment and 7 to 12 cm water phase were taken from box core samples and transferred in thermoboxes to the laboratory within 4 h. Bottom water for incubation was pumped from 0.5 m above the bottom and transported in plastic
containers. Winkler samples for determination of O₂ concentration in the bottom water were also taken. In the laboratory the sediment cores were opened and immersed in an aquarium with 20 l bottom water thermostatted at the in situ temperature (±1°C). The water column in each core was mixed by small magnetic stirrers driven by an external magnet (30 r.p.m.). This stirring rate created the same diffusive boundary layer thickness of about 400 µm as in the flux chamber. The O₂ concentration was maintained near in situ levels by gentle purging with a N₂/air gas mixture. Incubation was initiated after about 4 h by mixing 15 mM I₅NO₃⁻ stock solution into the aquarium to a final concentration of 55 to 76 µM. The cores were closed with rubber stoppers after 15 min when complete mixing of bulk water with the water column of each core was ensured.

For testing of NO₃⁻ concentration effects I²NO₃⁻ was added to each individual core to obtain a range of concentrations from 2 to 60 µM I₅NO₃⁻. Samples for initial O₂ and NO₃⁻ concentrations were taken from the aquarium. Incubation times of 3 to 5.5 h ensured that O₂ was never depleted by more than 25% in any core. After incubation the stoppers were removed, water samples for O₂, NO₃⁻ and I₅N₂ were rapidly taken as previously described and 250 µl ZnCl₂ solution was added to each core. Sediment and water phases were mixed and a slurry sample was taken as previously described. Sediment was sieved and infauna collected.

Analysis and calculations. Nitrate was analyzed using the standard method (Grashoff 1983) and O₂ was analyzed by Winkler titration. One ml of the water in the Exetainers was exchanged with He, and dissolved N₂ was extracted in the headspace by vigorous shaking. Subsamples of the headspace were withdrawn with syringe and injected into a gas chromatograph in line with a mass spectrometer as described by Nielsen (1992). Water and slurry samples from cores without I₅N added were used as blanks and excess I₂N₄ and I₅N₂ were used to calculate the ratio of I₅N₄ paired with I₂N₄ versus I₂N₄ paired with I₅N₂ assuming uniform mixing and random pairing of the isotopes in the denitrification zone (Nielsen 1992):
Fig. 2. Comparison of indigenous denitrification rates (d14) estimated from in situ incubations in a benthic flux chamber and laboratory incubations of sediment core samples. Hatched portions of columns represent denitrification of NO3\textsuperscript{-} from the water column (d14w). Error bars indicate standard deviation for cores (laboratory, n = 6) or subsamples in the flux chamber (in situ, n = 3).

... contribution of d14w to d14 was only 8 μmol m\textsuperscript{-2} h\textsuperscript{-1}, which could be ignored.

In situ and laboratory incubations indicated similar rates of denitrification in the sea bottom both in June and November (Table 1, Fig. 2). More than 80% of the labelled N\textsubscript{2} produced during the incubations accumulated in the pore water, the rest being released to the water column (data not shown), and identical standard deviations were found for the cores incubated in the laboratory and for the similar sized subsamples taken in the flux chamber (Fig. 2). Denitrification of NO3\textsuperscript{-} produced by nitrification within the sediment was similar in June and November and the increased total denitrification rate in November was due to denitrification of NO3\textsuperscript{-} present in the water column in November (Table 1).

Oxygen consumption rates were 40 to 50% lower in the laboratory compared to those in situ (Table 1). The average O2 concentration in the laboratory cores was 20% higher than those in situ in June and 20% lower in November (Table 1).

Table 1. Incubation conditions and measured rates of O2 uptake and denitrification in situ and in the laboratory on June 10 and November 24, 1992

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Temperature (°C)</th>
<th>Salinity (%)</th>
<th>O2 (μM)</th>
<th>NO3\textsuperscript{-} (μM)</th>
<th>O2 Flux (mmol O2 m\textsuperscript{-2} d\textsuperscript{-1})</th>
<th>d14 (μmol N m\textsuperscript{-2} d\textsuperscript{-1})</th>
<th>d14n (μmol N m\textsuperscript{-2} d\textsuperscript{-1})</th>
<th>d14w (μmol N m\textsuperscript{-2} d\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun 10</td>
<td>In situ</td>
<td>7.5</td>
<td>26</td>
<td>95</td>
<td>0</td>
<td>16.0</td>
<td>348</td>
<td>348</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>7.5</td>
<td>26</td>
<td>115</td>
<td>0</td>
<td>8.5</td>
<td>291</td>
<td>291</td>
<td>0</td>
</tr>
<tr>
<td>Nov 24</td>
<td>In situ</td>
<td>9.6</td>
<td>28</td>
<td>201</td>
<td>8</td>
<td>17.7</td>
<td>474</td>
<td>303</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>9.8</td>
<td>28</td>
<td>160</td>
<td>8</td>
<td>10.3</td>
<td>500</td>
<td>371</td>
<td>129</td>
</tr>
</tbody>
</table>

DISCUSSION

Important assumptions of the isotope pairing method were supported by the experiment with different additions of 15NO3\textsuperscript{-} to sediment cores. The linear correlation of d15w and 15NO3\textsuperscript{-} concentration showed that denitrification of NO3\textsuperscript{-} from existing sources is not impeded significantly by addition of extra sources within the investigated concentration range. The independence of added 15NO3\textsuperscript{-} was also confirmed by the relatively constant estimate of the rate of denitrification of 14NO3\textsuperscript{-} (d14) for 15NO3\textsuperscript{-} concentrations above 10 μM. Microsensor and model studies have also shown that diffusional supply of NO3\textsuperscript{-} from the oxic zone rather than kinetics of denitrification within the anoxic zone is controlling the process (Christensen et al. 1990, Nielsen et al. 1990).

A key assumption for the calculation of d14 is a uniform mixing and preservation of the nitrogen isotopes over the denitrification zone and during the whole incubation (Nielsen 1992). Any non-uniform mixing will increase the formation of homogenous isotope pairs, 14N14N and 15N15N, relative to the mixed pair 14N15N and d14 will be underestimated by the standard calculation. Non-uniform mixing will occur in heterogeneous sediments with different spots having different activity of nitrification and denitrification. Isotopic dilution of 15NO3\textsuperscript{-} in the water phase due to efflux of 14NO3\textsuperscript{-} from the sediment during incubation also causes non-uniform isotope mixing. Bioturbation may similarly induce spatial and temporal heterogeneity in isotope mixing. The actual significance of non-uniform isotope mixing, however, is readily tested by incubations with a range of 15NO3\textsuperscript{-} concentrations as done in this study. Higher concentrations of 15NO3\textsuperscript{-} minimize the potential underestimation of the denitrification of 14NO3\textsuperscript{-} (d14) because a larger fraction of the 14NO3\textsuperscript{-} being denitrified is paired with 15NO3\textsuperscript{-} and directly measured as 14N15N, while less ends up as 14N14N which is the indirectly estimated part of d14 (Nielsen 1992, Pelegri et al. 1994, Rysgaard et al. 1995). Underestimates of d14 at low 15NO3\textsuperscript{-} levels is indeed demonstrated by the data in Fig. 1 where the calculated d14...
rate drops off at $^{15}$NO$_3^-$ concentrations below 10 $\mu$M. The calculated d14 rate does not increase at concentrations above 10 $\mu$M, thus confirming that the standard levels of 20 to 80 $\mu$M $^{15}$NO$_3^-$ used here and in other studies (Nielsen 1992) eliminated the effect of non-uniform isotope pairing. A similar concentration test in a very intensively bio-ventilated estuarine sediment suggested $^{15}$NO$_3^-$ concentrations between 60 and 300 $\mu$M to be applied for optimal determination of d14 (Pelegri et al. 1994). After injection of $^{15}$NO$_3^-$ it takes some time before diffusion has established a steady state $^{15}$NO$_3^-$ profile, and it is important that this period is short as compared to the total incubation time. In our case the O$_2$ penetration depth was less than 2.5 mm (J. Gundersen unpubl. data), which ensured that an equal distribution of the 2 species of NO$_3^-$ was established relatively shortly after injection. In any case, the test of uniform isotope mixing discussed above will also indicate if establishment of the NO$_3^-$ profiles is a problem.

The similar rates of denitrification and the low standard deviations (Fig. 2) obtained by measurements in situ and in the laboratory were promising and indicated that both the denitrification process and the method are relatively steady. Blackburn & Henriksen (1983) calculated denitrification rates from differences of nitrification rates and NO$_3^-$ fluxes at a nearby station in the bight. They estimated rates of 500 and 300 $\mu$mol m$^{-2}$ d$^{-1}$ in July and November 1979. These rates are quite similar to the rates reported here. Jensen et al. (1988) measured denitrification at 3 other stations in the bight using the acetylene block technique and the rate estimates varied considerably, from 90 to 800 $\mu$mol N m$^{-2}$ d$^{-1}$ in July 1985 and from 60 to 280 $\mu$mol N m$^{-2}$ d$^{-1}$ in November 1985. Acetylene also blocks nitrification and Seitzinger et al. (1993) showed that in a lake sediment the technique could not capture coupled nitrification-denitrification. The NO$_3^-$ concentration in the bottom water in July 1985 was 8 $\mu$M (M. H. Jensen pers. comm.) and the denitrification activity measured by the acetylene block technique might in this case be ascribed to diffusion of NO$_3^-$ from the water column. In November 1985, however, the NO$_3^-$ concentration was 4 $\mu$M and yet the denitrification estimates were significant. In November 1985 a deeper O$_2$ penetration may imply that the pool of NO$_3^-$ in the sediment arising from nitrification is larger and may contribute significantly to the denitrification activity after inhibition of nitrification. The efficiency of the acetylene block technique under varying conditions still needs further investigations.

The dominating infauna at the station was the bivalve Abra alba (400 ind. m$^{-2}$ with an average length of 15 mm in June, and 300 ind. m$^{-2}$ with an average length of 10 mm in November). The higher O$_2$ uptake rates measured in situ as compared to the rates measured in the laboratory were partly caused by inactivation of the infauna upon sampling and partly by lower density of animals in the sampled cores. It was observed that only a few of the bivalves in the cores had their siphons extending to the surface and were thereby actively respiring. This was generally observed when cores for incubations were brought back to the laboratory (J. Gundersen & R. Glud unpubl.). The burred bivalves pump respiration water through siphons extending to the sediment surface and the sediment surrounding the siphon may not be oxygenated or otherwise affected. Therefore no differences in denitrification activity were observed between the laboratory and the in situ incubation even though the O$_2$ uptake in situ was significantly higher (Table 1). Some burrow dwelling animals are known to stimulate nitrification and denitrification considerably by pumping water with O$_2$ and NO$_3^-$ into the sediment (Henriksen et al. 1980, Aller 1988, Kristensen et al. 1991) and if collection of sediment cores impedes their activity, gross underestimates of in situ denitrification activity might be obtained by laboratory incubations.

This study was performed at a shallow site (16 m) where pressure changes during recovery are not likely to influence biological processes. At larger depths, where decompression during sediment sampling may disturb biological activity and porewater chemistry (Jahnke et al. 1989, Glud et al. 1995), the combination of in situ flux chambers and isotope pairing technique presented here seems optimal for measuring denitrification. With increased O$_2$ penetration depth it takes more time to establish a steady state gradient of $^{15}$NO$_3^-$ in the sediment. The incubation time and the height of the enclosed water phase therefore have to be increased in parallel with O$_2$ penetration depth. Heating of sediment cores during recovery can affect measured metabolic rates and porewater profiles (Glud et al. 1995). In situations where a transient heating of the sediment cores can be expected either in the water column or during extended recovery time or time on deck, injection of ZnCl$_2$ into the incubation chamber can be performed before recovery. The in situ technique may also be optimal in many limnic systems with surface located denitrification where ebullition of methane bubbles makes it impossible to sample undisturbed sediment cores even from shallow depth.

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LITERATURE CITED


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