Seasonal variation in denitrification rates and nitrous oxide fluxes in intertidal sediments of the western Wadden Sea

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ABSTRACT: Variation in denitrification rates, nitrous oxide fluxes and nitrate concentration of the overlying water were studied over 1 yr in sediments from the intertidal zone in the western Wadden Sea, The Netherlands. Denitrification rates were highest in winter and early spring, while in summer the rates were low. Maximum rate was 55 μmol N m^{-2} h^{-1}; minimum rate was 1 μmol N m^{-2} h^{-1} N$_2$O fluxes were from the sediment to the water column in winter and early spring (maximum 0.6 μmol N m^{-2} h^{-1}), while in summer fluxes from the water column to the sediment were recorded (maximum -0.3 μmol N m^{-2} h^{-1}). For marine systems this is the first time that N$_2$O influxes into the sediment over a considerable part of the year are reported. On an annual basis the denitrification rate and N$_2$O flux were 110 mmol N m^{-2} yr^{-1} and 0.3 mmol N m^{-2} yr^{-1} respectively. Denitrification rates and NO$_3^-$ fluxes were coupled to the NO$_3^-$ concentration in the overlying water, as confirmed by NO$_3^-$ enrichment experiments.

INTRODUCTION

Denitrification is an important process in the nitrogen cycle. In the absence of oxygen, nitrate and nitrite are used as terminal electron acceptors for respiration and are converted to nitrous oxide and/or nitrogen gas (Fenchel & Blackburn 1979, Knowles 1982):

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$
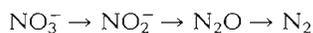
The ratio of N$_2$O to nitrogen gas production is controlled by factors such as oxygen concentration (Betlach & Tiedje 1981, Jørgensen et al. 1984), presence of hydrogen sulfide (Sørensen et al. 1980), NO$_3^-$ concentration (King & Nedwell 1987), availability of organic matter and composition of the microbial population (King & Nedwell 1985). Denitrification is a process which has the potential to counteract the increasing nitrogen load to coastal systems. It can, however, result in the emission of N$_2$O to the atmosphere, where it is involved in global warming (greenhouse effect; Wang et al. 1976) and destruction of the ozone layer (Liu et al. 1977, Crutzen 1981). The global input of N$_2$O to the atmosphere is estimated to be 14 Tg N yr^{-1}, and the contribution of oceans and estuaries is ca 14 % (Seiler & Conrad 1987).

The presence of nitrate, and the limited oxygen penetration as a consequence of mineralization, make sediments of coastal systems favourable sites for denitrification. The aim of this research was to study seasonal variations in denitrification rates and N$_2$O fluxes across the water/sediment interface in a coastal system, as a function of NO$_3^-$ availability, temperature, and oxygen penetration.

MATERIALS AND METHODS

Sampling site. Samples were collected from the intertidal zone on the island of Texel, The Netherlands. The site, ca 10 m^2 was located near 'Marsdiep', an inlet between the North Sea and the western Wadden Sea (Fig. 1). The sediment can be characterized as sandy with a silty surface layer (1 to 2 cm). The organic C content of the surface layer was 0.3 to 0.5 % dry wt. The macrofauna was dominated by *Arenicola marina*, *Lanice conchilega* and *Heteromastus filiformis*.

Samples were taken at low tide every 2 to 3 wk. Plexiglas tubes (i.d. = 5.4 cm, length = 25 cm) were inserted into the sediment, which was still covered with 10 to 20 cm of water. The cores were stoppered with rubber bungs, and samples from the overlying water were collected in polyethylene bottles. Salinity of the overlying water, which varied from 28.5 to 31.5 ‰, was
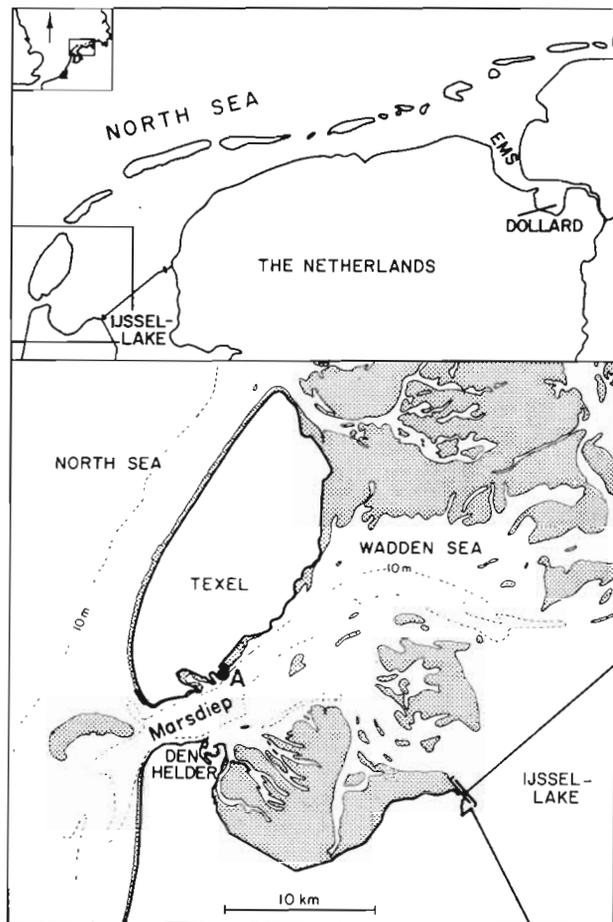
Fig. 1. Location of sampling site (A) in the western Wadden Sea, The Netherlands. Shaded areas represent intertidal flats

measured with a temperature-compensated salinometer (Kent Lab, USA).

Denitrification rates and N_2O fluxes. Within 1 h after sampling, 6 cores were prepared for measuring denitrification rates and N_2O fluxes. The sediment surface in the cores was adjusted to a fixed position, resulting in a 100 ml water volume and a 15 ml headspace volume. The cores were closed with gas-tight polyacetate lids, provided with a sample port and a teflon-coated magnetic stirrer.

Three cores were treated with acetylene (Andersen et al. 1984, Smith et al. 1985). To obtain a final acetylene concentration of 10 % (v/v), 10 ml of the water column was replaced by acetylene-saturated overlying water, and 200 µl of acetylene-saturated North Atlantic surface water (nitrate/nitrite-free, diluted with aqua dest to salinity of 30 ‰) was injected into the sediment through silicone-sealant-filled sideports, located at 0.5 cm depth intervals.

The 3 remaining cores were used for N_2O flux estimates.

All cores were incubated in the dark at in situ tem-

perature for ca 5 to 6 h. Gas samples (0.5 ml) were taken at fixed time-intervals from the headspace for N_2O analysis and replaced by equal volumes of pure nitrogen gas. The maximum and initial slope of N_2O change in water and gas phase were used to calculate denitrification rates and N_2O fluxes respectively. This procedure, according to Oremland et al. (1984) and Law (1989), was adapted after control experiments had indicated that in our type of sediment, with an oxygen penetration of only a few millimeters (Fig. 2), the increase of N_2O upon acetylene addition was restricted to the upper 0.5 cm and reached a steady state after 2 h of incubation. Over 95 % of the total N_2O amount was at this time present in the overlying water and in the head space.

Once in summer and once in winter, NO_3^- and lactate/acetate, the latter being common products of fermentation and well-documented substrates for denitrification, were added to the overlying core water to determine the possibility of their being a limiting factor in denitrification as well as in N_2O production. The NO_3^- concentration of the overlying water was raised 10-fold and lactate/acetate was added to a final concentration of ca 300 µM.

Oxygen profiles. Within 30 min after sampling, sediment oxygen profiles were determined in cores according to Revsbech et al. (1980) and Helder & Bakker (1985). Vertical profiles were recorded using an oxygen micro-electrode (Diamond Electro Inc., Ann Arbor, Michigan, USA; type 723) mounted on a micromanipulator, without stirring the overlying water.

Porewater analyses. Two cores were taken for nutrient profiles. The upper 2 cm was sliced in intervals of 0.5 cm; the remaining sediment (up to 10 cm) was sliced in 1 cm intervals. Slices of corresponding intervals were pooled and transferred into Reeburgh-type squeezers (Reeburgh 1967), and N_2 pressure was applied directly onto the sediment. Porewater and in situ overlying water were filtered with 0.2 µm cellulose acetate filters (Gelman, USA). The filtrate was collected and stored in polyethylene bottles at −5 °C. After thawing, samples were analyzed for NO_3^-, NO_2^- and NH_4^+ on an autoanalyzer system. NO_3^- and NO_2^- were determined according to Strickland & Parsons (1972); NH_4^+ determination followed the method described by Helder & de Vries (1979).

N_2O analyses. All gas samples were analysed for N_2O on a Packard 438a gas chromatograph equipped with an electron capture detector, which operated at a temperature of 310 °C; the inlet and column temperatures were 110 and 50 °C respectively. The column was Hayesep S (80 to 100 mesh, 2 mm × 2 m), and a pre-column filled with 0.5 cm³ Drierite and 0.5 cm³ Carbosorb was mounted in line to remove water vapor and CO_2 (Law & Owens 1990). A split system was used

for samples containing acetylene to prevent this gas from entering the detector.

Concentration of N_2O in water was calculated by using the solubility coefficient (F) of N_2O in seawater (Weiss & Price 1980). All N_2O samples were run against N_2O standards (90, 540, 1200 ppb; accuracy $\pm$ 5%; Scotty, Scott Specialty Gases, South Plainfield, New Jersey, USA).

RESULTS

Seasonal variation of the oxygen penetration depth in the sediment from March 1989 to April 1990 is given in Fig. 2A. The penetration depth varied from 1 mm in summer to 4.5 mm in winter.

In Fig. 2B the seasonal variation in denitrification rates and NO_3^- concentration of the overlying water are shown. Denitrification rates were low in summer ($<$ 2.5 μmol N m^{-2} h^{-1}) and high in winter (10 to 60 μmol N m^{-2} h^{-1}). In summer, when NO_3^- concentrations were low ($<$ 2.5 μM), denitrification rates were correspondingly low. In late summer, when NO_3^- concentrations in the overlying water increased up to 80 μM, denitrification rates also tended to increase, although in an irregular pattern. The drop in NO_3^- concentration in April 1989 coincided with the spring phytoplankton bloom.

Seasonal variation in N_2O flux across the sediment-water interface is shown in Fig. 2C. Fluxes of N_2O also seemed to be related to NO_3^- concentrations and (directly or indirectly) to denitrification rates. In summer, when NO_3^- concentrations in the overlying water were low, fluxes of N_2O from the overlying water to the sediment were observed (influxes). In winter, when NO_3^- concentrations in the overlying water were relatively high, the sediment behaved as a source of N_2O. The data from January 1990 are an exception to this rule, probably due to severe storms for several days preceeding sampling, by which porewater gradients were disturbed and surface sediments were resuspended.

In winter, no significant effect was observed on denitrification rates after addition of NO_3^- to the overlying water (Fig. 3A), while in summer NO_3^- addition resulted in a strong increase (Fig. 3B).

Addition of lactate/acetate to the overlying water did not have any significant effect in either summer or winter (not shown). The effects of NO_3^- and lactate/acetate additions on N_2O fluxes were similar to their effects on the denitrification rates; in winter, addition of NO_3^- did not result in an increase of N_2O flux from the sediment to the overlying water (Fig. 4A), while in summer a strong increase was observed (Fig. 4B). Neither in winter nor in summer was any effect observed when lactate/acetate was added (not shown).
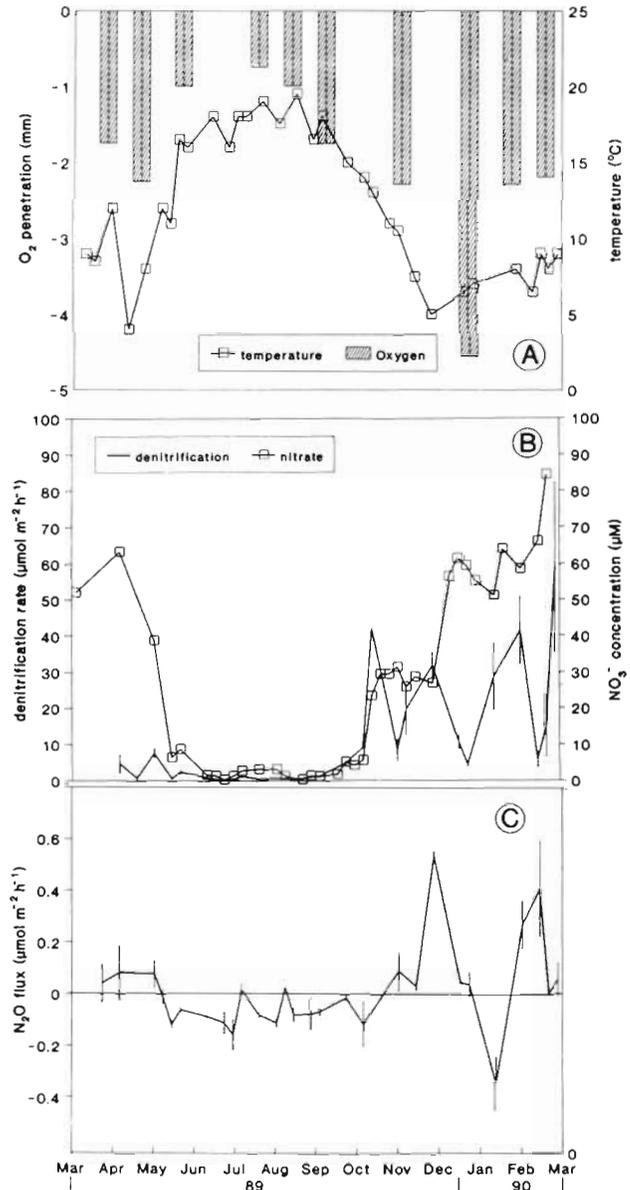

Fig. 2. Annual patterns of (A) oxygen penetration depth in the sediment and temperature of the overlying water; (B) sediment denitrification rates and NO_3^- concentration of the overlying water; (C) N_2O fluxes. Positive values indicate fluxes from the sediment to the overlying water. Vertical bars represent SD (n = 3)

Porewater profiles of NO_3^- were recorded throughout the year. In late summer NO_3^- concentration in the porewater was relatively low ($<$ 3 μM) at all depths. In winter the concentration in the upper few centimeters increased to concentrations $>$ 30 μM (Fig. 5).

DISCUSSION

Denitrification is an anaerobic process which depends, apart from temperature and the availability of
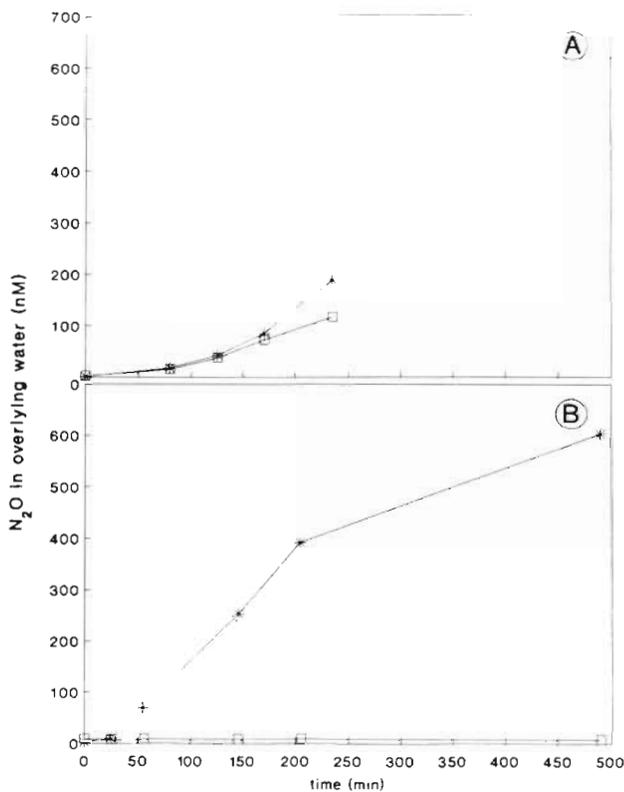
Fig. 3. Effect of nitrate enrichment on denitrification rates. (A) January 1990. (–*–) Denitrification at an NO_3^- concentration in the overlying water of 660 μM; (–□–) denitrification at ambient NO_3^- concentration (66 μM). (B) July 1989. (–*–) Denitrification at an NO_3^- concentration in the overlying water of 35 μM; (–□–) denitrification at ambient NO_3^- concentration (3.5 μM)

NO_3^- in overlying water is at low concentration (< 3.5 μM) in late spring and in summer, due to its depletion by phytoplankton, and reaches relatively high concentrations (> 80 μM) in winter (Fig. 2B). The seasonal change in NO_3^- concentration of overlying water is reflected in the porewater profiles (Fig. 5). An important feature of these porewater profiles is that they do not demonstrate a distinct maximum near the sediment-water interface, which would have been indicative of nitrification. Although the absence of these maxima may be partly due to the restricted vertical resolution (0.5 cm), it seems that downward diffusion of NO_3^- from the overlying water is responsible for the NO_3^- supply to the denitrification layer, and that close coupling between nitrification and denitrification (Jenkins & Kemp 1984) does not occur. This is in line with the results summarized in Fig. 3. In summer, when ambient NO_3^- concentration in the overlying water was low (3.5 μM), addition of NO_3^- resulted in a considerable increase (> 100-fold) in denitrification rate (Fig. 3B), while in winter at an ambient NO_3^- concentration of 66 μM such an effect was nearly absent (Fig. 3A). These

suitable organic substrates, on a niche where NO_3^- is still present but where oxygen is virtually absent. The seasonal variation in oxygen penetration which we observed at our sampling site (Fig. 2) thus would force the denitrification zone to move in response to the changing oxygen penetration depth (Nielsen et al. 1990). Overall denitrification rate is also dependent on the thickness of the aerobic layer, which acts as a diffusion barrier for NO_3^- from the overlying water or from the oxygenated surface sediment layer, where NO_3^- can be produced by nitrification (Nielsen et al. 1990). Although this mechanism was probably active during the period of our measurements it was not obvious, as can be concluded by comparison of Fig. 2 A & B, which do not indicate a relation between oxygen penetration depth and denitrification rate. Other mechanisms must have been more important.

Availability of suitable organic substrates for denitrification was not an important factor, because the addition of lactate and acetate influenced neither in summer nor in winter the measured denitrification rates.
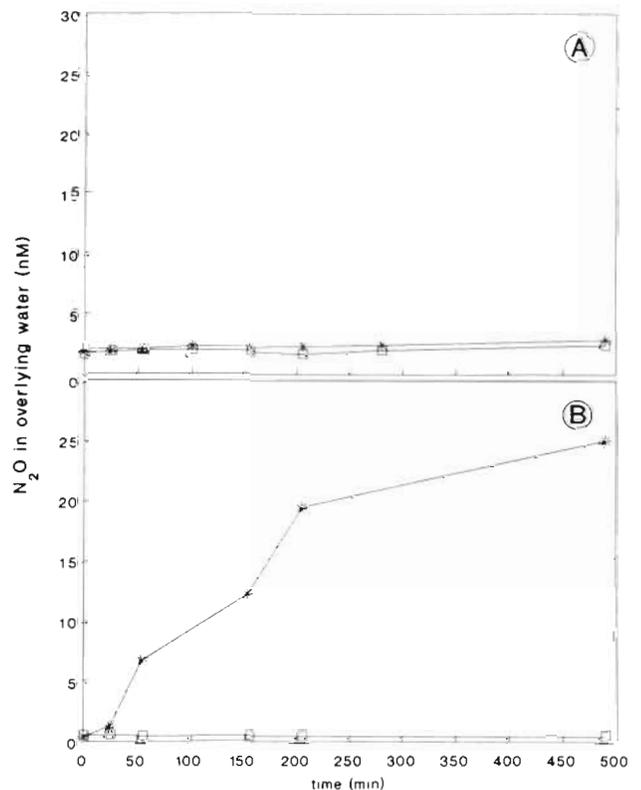

Fig. 4. Effect of nitrate enrichment on nitrous oxide fluxes. (A) January 1990. (–*–) N_2O evolution at a NO_3^- concentration in the overlying water of 660 μM; (–□–) N_2O evolution at ambient NO_3^- concentration (66 μM). (B) July 1989. (–*–) N_2O evolution at an NO_3^- concentration in the overlying water of 35 μM; (–□–) N_2O evolution at ambient NO_3^- concentration (3.5 μM)
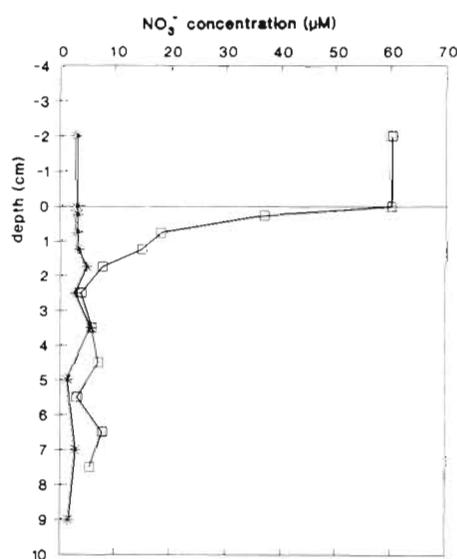
NO$_3^-$ concentration (µM)

Fig. 5. Porewater profiles of nitrate concentration (µM). (−∗−) Summer, August 1989; (−□−) winter, January 1990

observations suggest that in summer the ambient NO$_3^-$ concentration is below, though in winter well above, the half-saturation value (K_m) for denitrification, which is usually in the range of 2 to 15 µM (Murray et al. 1989, Rehr & Klemme 1989). Once the NO$_3^-$ supply from the overlying water to the denitrification zone increases again (October), other mechanisms are responsible for the considerable variation in denitrification rates in winter and early spring.

The minimum and maximum denitrification rates were ca 1.0 and 50.0 µmol N m^{-2} h^{-1} respectively. Compared with values summarized by Seitzinger (1988) and Koike & Sørensen (1988), the present data are on the lower end of the range, though similar to data given by these authors for the coastal North Sea and the Kysing Fjord (Denmark). On an annual basis the average denitrification rate for our sampling site is 110 mmol N m^{-2} yr^{-1}.

Although we did not measure the relative importance of denitrification in overall NO$_3^-$ turnover in the anoxic sediment layer, data from a similar site nearby indicate that ca 63 % of the added ^{15}N-NO$_3^-$ is denitrified to N$_2$, while 32 % ended up as NH$_3$ (Goeyens et al. 1987).

The observed seasonal variation in denitrification rates at our sampling site resembles the pattern described by Jensen et al. (1988). In coastal sediments from the Aarhus Bight (Denmark), a slow decrease in denitrification activity from early spring until late summer was observed by these authors. The minimum denitrification rates were 2 to 8 µmol N m^{-2} h^{-1}; maximum rates were observed in spring (15 to 40 µmol N m^{-2} h^{-1}). Jensen et al. (1988) suggest that NO$_3^-$ production from NH$_3$ originating from mineralisation of

detritus which is formed in the spring bloom, could enhance denitrification. Alternatively, a stimulation of mineralisation could result in a decrease in oxygen penetration into the sediment, thereby repressing the nitrification rate but reducing the diffusive barrier for NO$_3^-$ from the overlying water. In the latter case denitrification in the sediment might depend on NO$_3^-$ concentration of overlying water, which seems in line with the observations at our sampling site.

As with all biological processes, denitrification is controlled by temperature (Smith et al. 1985, Law 1989), though the overall temperature effect may be limited by NO$_3^-$ availability (Sørensen 1984, Jørgensen & Sørensen 1985). In the present study, the temperature rise in May could have had a stimulating effect on denitrification rate, but this potential increase was counteracted by the simultaneous drop in NO$_3^-$ concentration. Thus, the net effect of annual cycles in NO$_3^-$ concentration and temperature is that maximum denitrification rates occur in fall and winter.

The N$_2$O fluxes observed in this study (− 0.15 to 0.55 µmol m^{-2} h^{-1}) are in the same range as those given by Seitzinger (1988) for coastal marine systems. Annual patterns in sediment-water exchange of N$_2$O have been reported for the Kysing Fjord (Denmark) by Jørgensen & Sørensen (1985). They found maximum N$_2$O fluxes of 2.5 to 5.5 µmol N m^{-2} h^{-1}, which were, as in our study, coupled with maximum denitrification rates in spring. On an annual basis net N$_2$O flux from the sediment to the overlying water at our site was 0.3 mmol N m^{-2} yr^{-1}. Given an annual averaged denitrification rate of 110 mmol N m^{-2} yr^{-1}, the ratio of N$_2$O emission from the sediment to denitrification rate is 0.27 %, which compares well with data for non-eutrophic coastal marine environments as summarized by Seitzinger (1988). A possible explanation for the flux of N$_2$O to the sediment during summer as reported here is the utilization of N$_2$O as a terminal electron acceptor for organic C degradation in the absence of NO$_3^-$ (Koike & Hattori 1975, Snyder et al. 1987). In summer, oxygen penetration in the sediment is limited (Fig. 2A) and NO$_3^-$ porewater concentrations are low (Fig. 5). This results in conditions favourable for use of N$_2$O as electron acceptor.

The phenomenon of N$_2$O influxes is observed in grasslands (Ryden 1981) and was described for marine systems by Jensen et al. (1984) in experiments on day/night variations of N$_2$O fluxes and denitrification rates in the Limfjorden (Denmark). Influxes were recorded during the light periods, and effluxes during the dark periods. According to the authors the efflux was caused by an increase in denitrification activity in the upper few millimeters as a result of decreased oxygen penetration into the sediment at night. In experiments with sediments from an intertidal flat in San Francisco Bay,

Miller et al. (1986) also observed N_2O influxes in intact cores and sediment slurries. Overall N_2O influxes did not occur in the presence of high NO_3^- concentrations, as this would have cause N_2O production which exceeded the consumption rate. According to the authors an alternative explanation would be a cessation in N_2O consumption as a consequence of the presence of a competitive electron acceptor (NO_3^-).

In a global perspective the N_2O fluxes at our study area must be categorized as very low. The highest fluxes of N_2O are reported from cultivated, fertilized soils, where N_2O efflux can amount to 1180 mmol N m^{-2} yr^{-1}. In natural aquatic and terrestrial environments highest N_2O fluxes were found in tropical, moist forests, where a range of 6.4 to 18.6 mmol N m^{-2} yr^{-1} was reported (Bouwman 1990).

Thus in conclusion, it seems that although coastal marine environments are a net source of N_2O on an annual basis, their ecological significance might be much more that they can act as a sink for atmospheric N_2O over a considerable period of the year.

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