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Occurrence of Nitric and Nitrous Oxides in a Coastal Marine Sediment

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The distribution of denitrification activity in a coastal marine sediment was determined by the acetylene inhibition technique and compared to concentration profiles of NO_3^-, NO_2^-, NO, and N_2O. The bulk of the denitrification activity was associated with the accumulation of NO_3^- in the oxidized surface zone of the sediment, but a secondary denitrification zone was occasionally found in the deeper layers where oxidized patches had been introduced by the burrowing activity of the macrofauna. Maxima of NO and N_2O were not associated with the peak activity of denitrification in the surface zone but were located at the lower edge of the activity profile. Significant accumulation of NO was found at the redox transition zone towards the deeper, sulfide-rich layers.

Both NO (nitric oxide) and N_2O (nitrous oxide) are gaseous products of bacterial denitrification. The biochemical pathway has not been conclusively established because of a controversy over the intermediates between NO_2^- and N_2, but the reaction sequence $NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$, originally proposed by Payne (11), is now most commonly accepted. The participation of N_2O was recently confirmed by the evidence of stoichiometric conversion of NO_3^- (and NO_2^-) to N_2O by denitrifying cultures in which the ultimate reduction of N_2O to N_2 was inhibited by acetylene (2, 18). The involvement of NO as an obligatory intermediate is still disputed (12).

Bacterial denitrification may be important for the production and consumption of NO and N_2O in natural environments, but the potential significance of other processes should be considered. Thus, the production of N_2O by nitrifying bacteria (16) and the reduction of N_2O by N_2-fixing species (4) are biological processes that may influence the occurrence of N_2O in certain habitats. Chemical processes may also generate nitrogenous gases. Accumulation of NO_2^- promotes NO and N_2O production by interactions between NO_2^- (and NO) and organic matter (14), and chemical dismutation of HNO_2 (nitrous acid) may be significant under acid conditions (10).

Several authors have presented profiles of N_2O concentrations in natural environments (3, 5, 7, 8, 13, 17). The field study of Dowdell and Smith (5) revealed an inverse relationship between the levels of N_2O and O_2 in the soil atmosphere, and the accumulation of N_2O was attributed to bacterial denitrification. A similar relationship was observed in deep-sea profiles of dissolved N_2O and O_2 (7, 17), but the possible origin of N_2O from sources other than denitrification was emphasized by Yoshinari (17). Significant production of NO in NO_3^--amended soils has been reported by Bailey (1), but, again, the mechanism of origin was ambiguous.

Direct assays for the processes that may generate NO and N_2O in natural environments should facilitate the interpretations of profile records. The present work reports the presence of significant concentrations of NO and N_2O in a coastal marine sediment and discusses the distribution and origin of these gases in relation to the measured activity of denitrification.

MATERIALS AND METHODS

The sediment samples were taken in clear Plexiglas tubes from the Kysing Fjord, a shallow, brackish basin on the east coast of Jutland, Denmark. Some cores were frozen on location, in liquid N_2, for subsequent analysis of the in situ concentrations of NO_3^-, NO_2^-, NO, and N_2O.

Other, unfrozen cores were used for an assay of denitrification activity. This assay was based on the acetylene inhibition technique and was previously described in detail (13). The cores were amended with acetylene-saturated water by injection and eventually frozen after 5 h of incubation in the dark at in situ temperature (2.5°C). Acetylene blocked the reduction of N_2O to N_2, and the accumulation of N_2O during incubation (denitrification activity) was measured in segments of the cores in a combined gas extraction and detection system. The gas extraction was performed in a helium-purged flask that contained the

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thawing segment for analysis, and the liberated N_2O (and NO) was trapped in a liquid N_2-cooled loop before injection into a gas chromatograph.

The extraction of NO and N_2O from a thawing 1-cm segment (5 cm^3 of sediment) was complete after 30 min of purge as measured by repeated cycles of the gas extraction procedure. Gas standards were injected directly into the helium line through a silicone rubber stopper on the sample flask. The standards were thus trapped and detected in the same way as the gases extracted from sediment samples. The recovery of NO from standards and sediment samples should be comparable even if NO was partially oxidized to NO_2 (nitrogen dioxide) by the presence of O_2 in the flask during the initial phase of the gas extraction. The recovery of NO by injection into the flask was 30% of that determined by direct injection into the gas chromatograph. The standard curve was linear for the 10- to 50-μl range, which covered the content of most of the sediment samples.

The methods of Strickland and Parsons (15) were applied to an automated analysis system (Chemlab) for the determinations of NO_3^- and NO_2^-. After gas analysis the sediment slurries were centrifuged at 2,000 $\times$ *g* for 15 min, and subsamples were taken from the supernatant for the assays.

The water content of the sediment was determined for the conversion of measured concentrations and activities into appropriate units, nanomoles of N per milliliter of pore water (micromolar) and nanomoles of N per milliliter of pore water per day, respectively.

RESULTS

The sediment was generally oxidized to a depth of about 5 cm as judged from the brown coloration (hydrous ferric oxides) and the positive redox potentials of the surface layer. The transition zone towards the deeper, reduced layers with negative redox potentials was usually only 1 to 2 cm wide. In such cores, the profiles of NO_3^-, NO_2^-, NO, and N_2O were regular, as shown by the selected core in Fig. 1. The concentration of NO_3^- declined rapidly with depth, from a maximum close to the sediment surface to low values at the transition zone. A small peak of NO_2^- was commonly observed in the oxidized zone, with peak concentrations located on the declining edge of the NO_3^- profile. Maxima of the gases, NO and N_2O, were invariably located at the transition zone. Peak concentrations of N_2O were always less than 5 μM, whereas the highest concentrations of NO were about 200 μM.

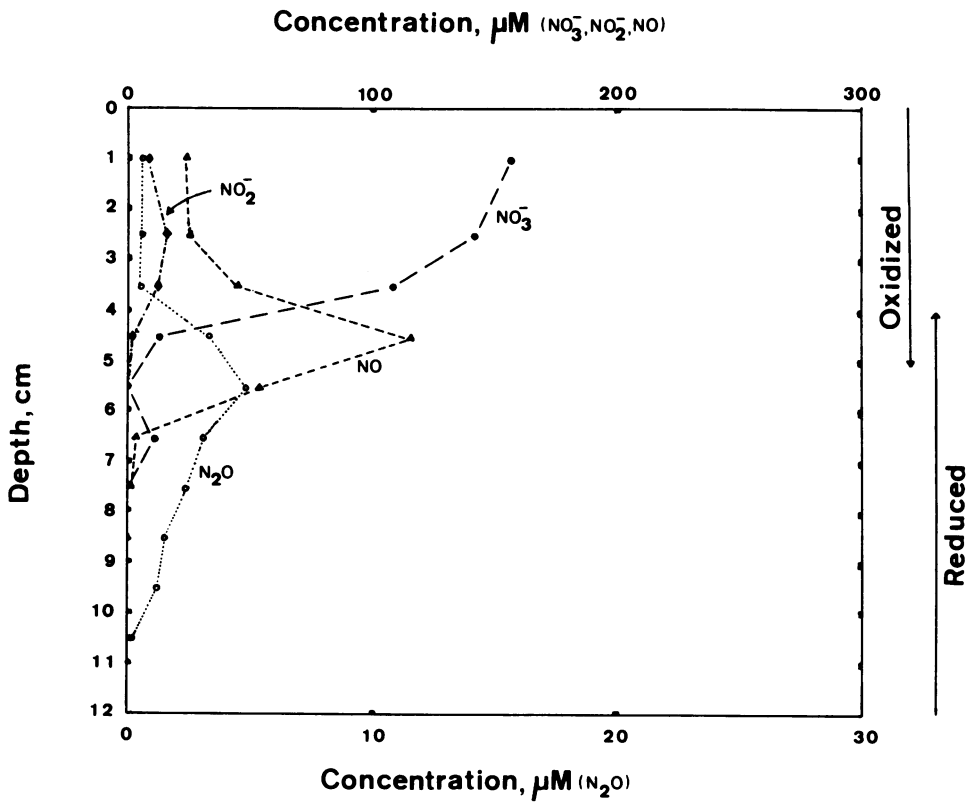

FIG. 1. *In situ concentrations of NO_3^-, NO_2^-, NO, and N_2O with depth in a sediment core without faunal burrows.*

Significant denitrification activity was associated with the high concentrations of NO_3^- in the oxidized surface layers of these cores, as shown by the selected core in Fig. 2. Peak activity was found at 1 to 3 cm below the sediment surface, and only a little activity was detected at the low concentrations of NO_3^- in the transition zone. The accumulations of NO and N_2O were apparently not associated with the main zone of denitrification activity, the maxima being located at the lower edge of the activity profile.

Other cores contained brown, oxidized patches in the black, sulfide-rich layers below the transition zone, apparently induced by the burrowing activity of the macrofauna—mainly polychaetes and lamellibranchs. A secondary denitrification zone was found in the deeper layers of such cores (see Fig. 3). This core also showed significant accumulation of NO in the heterogenous transition zone.

DISCUSSION

The nitrogen oxides involved in bacterial denitrification showed a sequence of maxima with depth in the sediment, but the activity of denitrification was most consistently related to the NO_3^- accumulation. Denitrification was apparently limited by the presence of O_2 in the upper 1 cm of the sediment. It is of interest that denitrification occasionally was significant in the deeper layers below the oxidized surface zone when NO_3^- was provided in oxidized patches around the faunal burrows. The presence of such secondary zones of denitrification in subsurface layers of marine sediments with an abundant macrofauna was recently deduced from pore water profiles of NO_3^-, NO_2^-, and NH_4^+ (6). The present work confirmed that the faunal activity may provide significant accumulations of nitrogen oxides and activity of denitrification in deeper layers.

The maxima of NO and N_2O were consistently related to the redox transition zone. In most cores only modest denitrification activity was found in this zone; if NO and N_2O were produced by bacterial denitrification, the accumulation of these gases was probably caused by some environmental parameter that inhibited further reduction. The high concentrations of NO as compared with the other nitrogen oxides are surprising. Nitric oxide can be adsorbed onto clay minerals (9), however, and if such adsorption was of

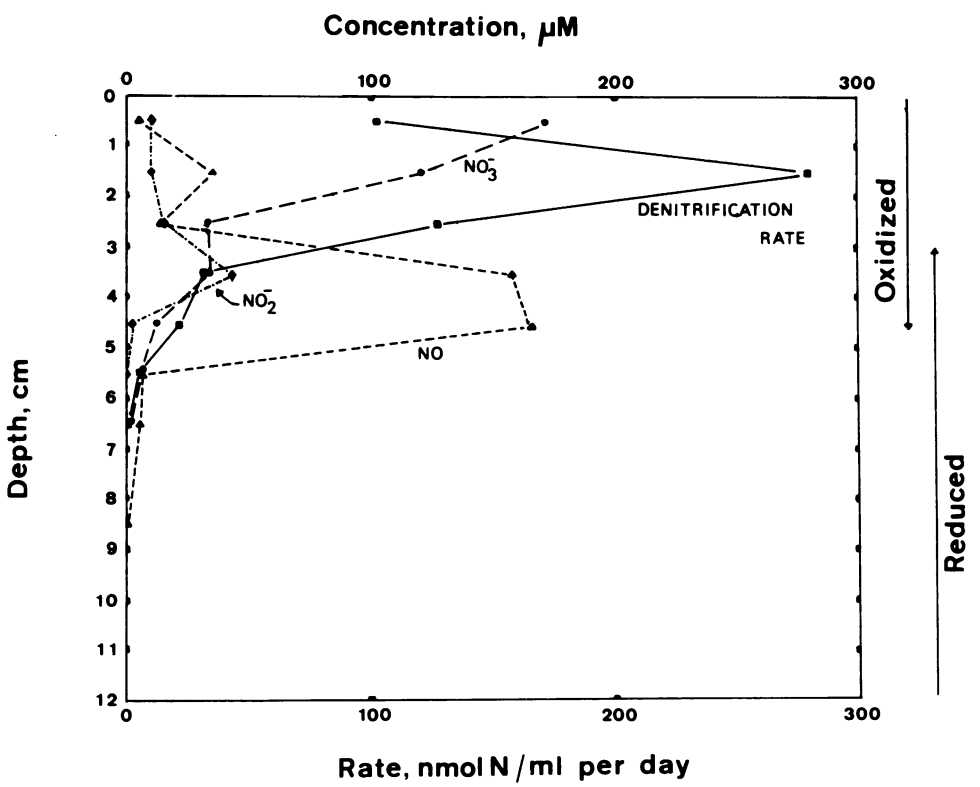

FIG. 2. *Denitrification activity and concentrations of NO_3^-, NO_2^-, and NO with depth in a sediment core without faunal burrows.*

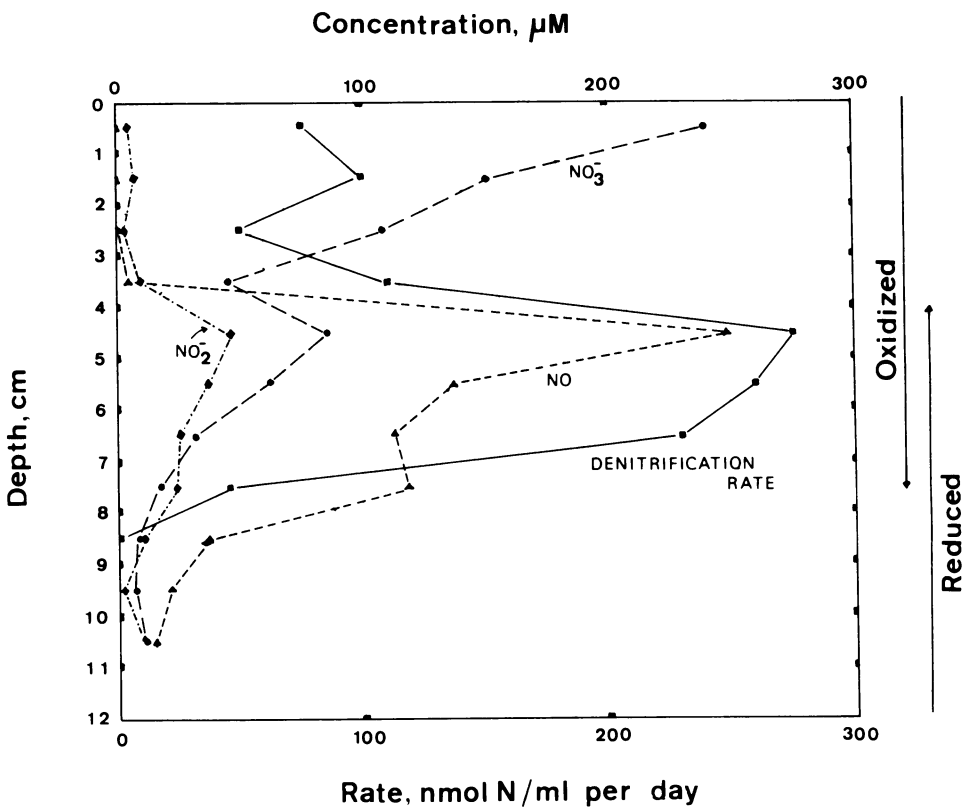

FIG. 3. *Denitrification activity and concentrations of NO_3^-, NO_2^-, and NO with depth in a sediment core with faunal burrows.*

significance in the sediment, the NO may not have been available for further biological reduction.

The data do not exclude the possibility that NO and N_2O were produced by chemical processes, but several features of the NO_2^- accumulations make the biological origin of these gases likely. (i) The maxima of NO and N_2O in the cores were not always coincident with any accumulation of NO_2^- (Fig. 1). (ii) Dismutation of HNO_2 is unlikely at the neutral-alkaline pH of this sediment. (iii) The reaction of NO_2^- with organic matter by nitrosation would seem to be of little importance at the low concentrations of NO_2^- in the sediment (less than 50 μM). In comparison, the NO_2^- level applied by Stevenson et al. (14) in their study of nitrosation in soil was as high as 0.15 M.

The results suggested that bacterial denitrification was the source of the NO and N_2O in the sediment. Both denitrification and sulfate reduction showed considerable activity at 2.5°C in this coastal marine sediment, but the processes showed a mutually exclusive pattern in regard to spatial distribution (Sørensen et al., submitted for publication). The present results indicated that the sulfate reduction may exert an influence on denitrification in the intermittent redox transition zone. It seems likely that the decreasing redox potential or the increasing sulfide concentration with depth may have caused the sequential accumulations of reduced nitrogen oxides.

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