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Emission of N_2O, nitrification and denitrification in a eutrophic lake sediment bioturbated by *Chironomus plumosus*

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ABSTRACT: Emission of nitrous oxide and rates of nitrification and denitrification were determined in sediment from a eutrophic lake in southern Sweden. Effects of bioturbation on nitrogen transformation were investigated by incubating sediment at 10 and 15°C in a continuous flow-through system containing ca 2000 tube-dwelling larvae of *Chironomus plumosus* L. (Chironomidae, Diptera) m^{-2}. N_2O emission was found to be independent of nitrate concentration (33 to 267 µM) in the overlying water at both 10 and 15°C. It is shown that *C. plumosus* larvae significantly enhanced the emission of N_2O from the sediment at 15°C, the highest emission recorded under these conditions was 0.45 ± 0.2 µmol N m^{-2} h^{-1} Since released N_2O was proportional to the rate of nitrification, it is suggested to originate mainly from this process. Bioturbation stimulated the rate of nitrification by a factor of 1.8 at 15°C. However, no enhancement of nitrification by chironomids was observed at 10°C In contrast to N_2O emission and nitrification, denitrification in both bioturbated and non-bioturbated cores was significantly correlated to the concentration of nitrate in the overlying water. The stimulation of denitrification by bioturbation at 10 and 15°C was 3.5- and 4.3-fold respectively. Denitrification rates were mainly accounted for (98%) by changes in the concentration of nitrate in the water, the water temperature and the biomass of *C. plumosus* larvae. *C. plumosus* larvae were found to decrease the N_2O:N_2 ratio at both temperatures. This was explained by the water pumping activity of the animals, mobilizing released N_2O to denitrifiers in or close to the animal burrows. The ratio of N_2O:N_2 did not exceed 0.5% in any of the treatments.

KEY WORDS: Nitrous oxide · Nitrification · Denitrification · Lake · Sediment · Bioturbation · *Chironomus plumosus*

INTRODUCTION

Nitrous oxide (N_2O) has gained much notoriety because of its important contribution as a greenhouse gas as well as for its adverse affect on the stratospheric ozone layer (Crutzen 1970, Wang et al. 1976). Atmospheric nitrous oxide concentration is currently increasing at a rate of 0.2 to 0.4% yr^{-1} (Khalil & Rasmussen 1983, Prinn et al. 1990) and both anthropogenic and natural sources have been identified (Bowden 1986).

Among the natural sources, several microbial processes are involved in the production of nitrous oxide in aquatic sediments, the 3 most important being nitrification, denitrification and dissimilatory ammonium production. In the nitrification process, ammonium is oxidised to nitrate with nitrite and nitrous oxide as intermediate compounds (Goreau et al. 1980). Denitrifying bacteria reduce nitrate to dinitrogen in which process nitrous oxide is the last intermediate which is finally converted to atmospheric gas (Knowles 1982). Dissimilatory production of ammonium (DNRA) involves the microbial reduction of nitrate to ammonium in which nitrite acts as an intermediate which, under certain conditions, can be converted to nitrous oxide in sediments (Smith & Zimmerman 1981) and in soils (Tiedje 1982). Seitzinger (1990) reviewed the few publications on measurement of nitrous oxide emission from aquatic sediments. Generally, she found that the flux of N_2O is below 2 µmol N m^{-2} h^{-1} and less than 1% of the N_2 production. In a study by Seitzinger & Nixon (1985), an increase in nutrient loading was found to enhance both the efflux of nitrous oxide and the ratio

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of N_2O:N_2 released to the water from the sediment. Thus, it seems that eutrophication might increase the load of N_2O to the atmosphere (Seitzinger et al. 1984, Seitzinger 1990). Several other factors are known to increase N_2O:N_2 ratio in the denitrification process, e.g. lowered pH and increased H_2S (Betlach & Tiedje 1981, Samuelsson et al. 1988, Firestone & Davidson 1989).

To decrease nitrogen transport to the sea in order to reduce eutrophication of coastal areas, substantial efforts have been made to restore and create ponds and wetlands. Such areas are known to have a capacity for nitrogen retention, where denitrification is considered to be the most significant process (cf. Leonardson 1994). However, a higher proportion of streams, ponds, lakes and wetlands for recycling of nitrogen also implies the risk of increasing the emission of nitrous oxide to the atmosphere. This is especially so since these environments will be supplied with nutrient-rich drainage water from agriculture and sewage treatment plants.

Since published data on nitrous oxide efflux, particularly from freshwater sediments, are very sparse, there is an urgent need for information contributing to the assessment of the relative importance of lakes and wetlands in nitrous oxide emission to the atmosphere (Samuelsson & Klemedtsson 1991). Bioturbation by tube-dwelling infauna may significantly increase the rate of sediment nitrification and denitrification as shown in both estuarine (Henriksen et al. 1980, Kristensen et al. 1991, Pelegrí et al. 1994, Pelegrí & Blackburn 1995a) and freshwater sediment (Chatarpaul et al. 1979, 1980, Pelegrí & Blackburn 1995b, Svensson & Leonardson 1996, Svensson 1997). The role of bioturbation in nitrous oxide emission from sediments has only been commented on by Law et al. (1992), referring to an unpublished study showing that polychaetes significantly increased N_2O flux from an intertidal sediment. Since bioturbation considerably influences both nitrification and denitrification, there is a need for further studies.

In this paper, a laboratory experiment with eutrophic lake sediment bioturbated by tube-dwelling *Chironomus plumosus* L. larvae is presented. The emission of nitrous oxide, nitrification and denitrification were determined from bioturbated and non-bioturbated sediment, using a continuous flow-through system. The influence of nitrate concentrations in the water phase at 2 different temperatures was studied.

MATERIALS AND METHODS

Sediment and chironomid preparation. Sediment was collected from the eutrophic Lake Sövdesjön (50° 34' N, 13° 40' E) in southern Sweden during October 1995. Surface sediment (0 to 20 cm) was taken with an Ekman grab in areas of 4 m water depth and was passed through a 2 mm sieve to remove invertebrates, larger detritus and algal aggregates. The sediment of this lake is of algal origin and highly organic. The average porosity (water content) of the top 3 cm was 94% and the organic content 36% (by dry weight). On the same sampling occasion, chironomids were collected from the same depth. The fauna was dominated by 4th instar *Chironomus plumosus* larvae with an average dry weight of 2.92 ± 0.34 mg ind.$^{-1}$ (mean ± SD). The sediment was stored in darkness in an open jar (66 l) without air bubbling at 4°C. The chironomid larvae were stored separately, at the same temperature, in small amounts of the unsieved sediment placed in continuously aerated buckets containing lake water. The range of the concentrations of nitrate and ammonium in Lake Sövdesjön over the year is 10 to 100 and 5 to 20 µmol N l^{-1}, respectively.

Experimental design and sampling. After the sediment had settled and stabilized for about 2 wk, 16 plexiglass cylinders (25 cm long, 4.4 cm inner diameter) were pressed down into the open jar sediment and temporarily closed by butyl stoppers. One by one, 7 to 9 cm long sediment cores were withdrawn and transported to a holding container where the overlying water phase was replaced by nitrate- and ammonium-free artificial lake water prepared according to Lehman (1980). The water volume above the sediment was 207 ± 9 ml. Each core was equipped with a rotating magnetic stirrer positioned on the cylinder wall at 5 to 6 cm above the sediment surface. The stirring did not resuspend the sediment. The open cylinders were then transferred to a continuous flow-through system with a central magnet (modified from Risgaard-Petersen et al. 1994). Incubations were performed at 2 temperatures, with 8 cores kept at 10°C and 8 cores at 15°C. Air-bubbled nitrogen-free artificial lake water was continuously supplied to the cores and passed through the system at a constant rate of 175 ± 13 ml h^{-1} using a peristaltic pump. All incubations were performed in darkness. After 1 d in the flow-through system, bioturbation was initiated by adding 3 larvae of *Chironomus plumosus* to 4 sediment cores at 10°C and 4 at 15°C. The remaining 8 cores were kept as controls at 10 and 15°C, respectively. The larvae immediately dug down into the sediment. The number of added chironomids corresponds to an abundance of about 2000 ind. m^{-2}, which is in the range normally found in eutrophic lakes. Abundances from 200 to several thousand chironomids m^{-2} of *C. plumosus* type are reported from the profundal sediment in lakes of varying trophic states (Thienemann 1954). All cores were left undisturbed for a period of 8 d during which oxygen and ammonium concentrations were followed to ensure that the magnetic stirring was sufficient to prevent ver-

tical oxygen gradients in the water column from being formed and that the systems were in a steady state with respect to bacterial processes. Following this period of pre-incubation, ^{15}N-labelled KNO_3 was added to the inflow water reservoir. The nitrate concentration was increased during the experimental period to give final concentrations of 33 (88% enrichment of $^{15}NO_3$), 67 (92%), 133 (96%) and 267 (98%) µM $^{15}NO_3$ in the inflowing water. Reservoir water at each concentration of nitrate was cycled to the cores for 2 to 3 d during which sampling was performed every 6 to 8 h. When increasing the $^{15}NO_3$ concentration the new concentration was always allowed to equilibrate in the sediment system for 6 to 8 h before the next sampling. Ammonium concentration in the reservoir water was set at 14 µM for all treatments. Water samples for oxygen concentration and nitrogen species analyses were collected with a 20 ml gas-tight glass syringe from the outflow water. The outflow tubing was made of chloropren rubber characterised by low gas conductivity. When sampling for oxygen and nitrogen was performed, this outflow tubing was mounted directly to the syringe. For nitrous oxide, samples of 10 to 12 ml were collected from the outflow water. The mounted tube was then replaced by a canula and the volume adjusted to 6.0 ml that without any air exposure was transferred to evacuated 12 ml exetainers and immediately frozen for later analysis of nitrous oxide with a gas chromatograph. Samples of 12 ml were transferred to identical 12 ml exetainers and kept for about 1 mo at 4°C until analysis of 15-nitrogen was made with a mass-spectrometer. The experiment was terminated by sieving the sediment of each core, and the total dry weight (105°C) of the chironomid larvae was determined. Only 1 chironomid larva out of 24 added was found to be dead.

Analysis. Dissolved oxygen concentrations were determined by Winkler titrations using a high precision automatic potentiometric titrator (Mettler TM DL21; 0.1 to 0.3% coefficient of variance; Granéli & Granéli 1991). Ammonium was determined following the method described by Chaney & Marbach (1962) and nitrate and nitrite according to Wood et al. (1967) on a Technicon Auto Analyzer II. Following thawing, samples for the analysis of nitrous oxide were vigorously shaken manually for 30 s, once they had reached room temperature (20°C). A subsample of 0.5 ml of the gas phase was then injected into a Varian 3300 gas chromatograph equipped with a stainless steel column (inner diameter 3.2 mm) containing 80/100 mesh Porapak Q and operated at 50°C. Argon with 10% methane was utilized as carrier gas and the column was connected to an electron capture detector (ECD) maintained at 310°C. The head-space analyses were corrected for the nitrous oxide dissolved in the liquid phase (Flett et al. 1976) by use of a Bunsen solubility coefficient of 0.92 and 0.78 (10 and 15°C, respectively; Weiss & Price 1980).

Water samples for denitrification measurements were analyzed according to the nitrogen isotope pairing technique (Nielsen 1992). Two ml of the 12 ml sample was replaced by helium. After vigorous shaking, 50 µl of the gas phase was injected in a gas chromatograph in line with a isotope-ratio mass spectrometer (Hewlett-Packard 4100 GCMS) for ^{15}N-labelled dinitrogen pairs ($^{14}N^{15}N$ and $^{15}N^{15}N$) formed by denitrification (Nielsen 1992). The $\%^{15}NO_3$ enrichment in the water phase of each core was determined by GCMS analysis after biological reduction to dinitrogen (Risgaard-Petersen et al. 1993).

Calculations. Nitrous oxide emission, oxygen consumption and ammonium efflux were calculated using the general flux equation, $F = (C_e - C_i)V/A$, where C_e and C_i are respectively the effluent and influent concentrations of the nutrients and gases, V is the flow rate and A is the surface area of the sediment.

The rates of denitrification per m^2 were estimated using the ^{15}N isotope pairing technique (Nielsen 1992). Production of single-labelled ($^{14}N^{15}N$) and of double-labelled ($^{15}N^{15}N$) dinitrogen pairs represents the respective net fluxes. These are used to calculate $d15$ and $d14$, which are the rates of denitrification of $^{15}NO_3$ and $^{14}NO_3$, respectively (Nielsen 1992):

$$d15 = (^{14}N^{15}N) + 2(^{15}N^{15}N)$$

$$d14 = [d15(^{14}N^{15}N)]/2(^{15}N^{15}N)$$

The rate of denitrification of the nitrate diffusing from the overlying water (d_w) was calculated from $d15$ and $\%^{15}NO_3$, where $\%^{15}NO_3$ represents the ^{15}N atom% of the reservoir water:

$$d_w = d15/\%^{15}NO_3$$

The rate of denitrification of the nitrate produced by nitrification (coupled nitrification-denitrification, d_n) was calculated as the following difference (Nielsen 1992):

$$d_n = (d15 + d14) - d_w$$

where the sum of d_w and d_n represents the total denitrification as measured in the core.

The rates of nitrification per m^2 were estimated from the isotope dilution of the labelled nitrate and total nitrification was further calculated by adding the gross unlabelled nitrate efflux (R) and the coupled nitrification-denitrification (d_n) as described by Rysgaard et al. (1993):

$$R = [C_i(e - i)/(0.366 - e)](V/A)$$

where C_i represents the inflow concentration of NO_3, e and i the ^{15}N-labeled fractions of NO_3 respectively in the effluent and influent water and 0.366 the ^{15}N content of the nitrified ammonia.

RESULTS

Ammonium efflux and O_2 consumption

Steady state in the ammonium efflux, found to be negative, occurred ca 140 h after the incubations of the sediment cores were initiated into the flow-through system (Fig. 1). Any significant differences between the treatments were however not found over that period. Between 140 and 230 h, when the main sampling for N_2O, nitrification and denitrification was conducted, the oxygen consumption was found to be stable. Average oxygen consumption at 10°C was 305 ± 30 µmol m^{-2} h^{-1} in bioturbated cores and 253 ± 15 in the non-bioturbated ones. At 15°C the oxygen consumption was found to be considerably higher; 496 ± 12 and 408 ± 25 µmol m^{-2} h^{-1} in bioturbated sediment cores and in non-bioturbated sediment cores, respectively. At both temperatures, oxygen consumption in cores with chironomids was significantly higher relative to cores without ($p < 0.05$, ANOVA), 20% at 10°C and 22% at 15°C. Increasing the temperature from 10 to 15°C enhanced oxygen consumption by 62% in bioturbated cores and 61% in non-bioturbated cores (Fig. 1).

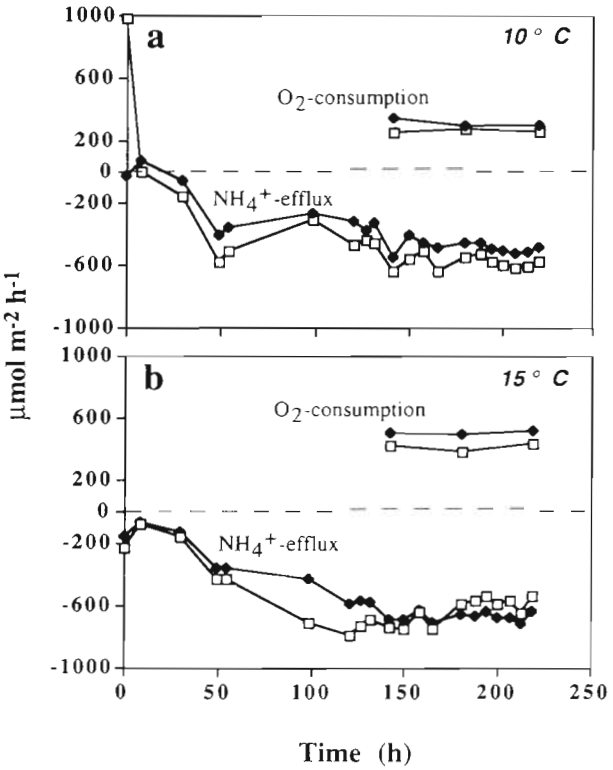

Fig. 1. Rates of ammonium efflux and oxygen consumption in eutrophic sediment from Lake Sövdesjön at (a) 10 and (b) 15°C in the presence (◆) and absence (□) of *Chironomus plumosus* (2000 ind. m^{-2}) versus sediment incubation time. Steady state conditions took place between 140 and 230 h. During this period sampling for N_2O, nitrification and denitrification was performed. Means for 2 separate cores are shown

Emission of N_2O

Nitrous oxide was found to be produced in the sediment in all treatments. Increasing emissions of N_2O from the lake sediment did not correlate with an increase in the nitrate concentration of the overlying water at 10 or 15°C in either bioturbated or non-bioturbated cores. However, there was a trend for the lowest flux of N_2O to be generally found at the lowest nitrate concentration in all treatments. The maximum flux was attained at nitrate concentrations between 50 and 150 µM in the overlying water in both bioturbated and non-bioturbated cores, at both temperatures.

Since no significant correlation between nitrate concentration in the overlying water and N_2O fluxes was established, the N_2O emissions associated to each experimental nitrate concentrations were expressed as mean emission (Fig. 2). At 10°C, bioturbation by chironomids did not significantly (ANOVA, $p > 0.05$) enhance N_2O emission from the sediment (Fig. 2). However, at 15°C, N_2O emission was found to be twice as high in bioturbated sediment as in controls without larvae (ANOVA, $p < 0.05$). Although slightly higher at 15 than at 10°C, average N_2O emissions were not significantly ($p > 0.05$) different in the controls.

Based on the average figures for N_2O emission observed in this experiment (Fig. 2), the annual emission from a eutrophic lake sediment with 2000 ind. m^{-2} of *Chironomus plumosus* larvae can be roughly estimated as 37 and 110 kg N km^{-2} yr^{-1}, assuming the predominant temperature over the year to be 10 and 15°C,

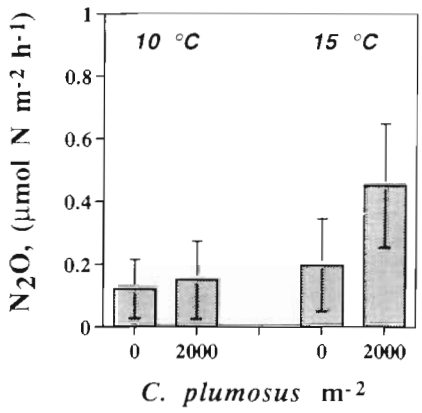

Fig. 2. Average emission of nitrous oxide in eutrophic sediment from Lake Sövdesjön at 10 and 15°C in the presence and absence of *Chironomus plumosus* (2000 ind. m^{-2}). Mean ± SD, n = 20 to 24

respectively. From a non-bioturbated sediment the N_2O emissions, although not significantly different, would be 30 and 48 kg N km^{-2} yr^{-1} at the predominant temperature of 10 and 15°C, respectively.

Nitrification

Nitrification rate showed similar patterns to those observed for the emission of N_2O. Hence, nitrification rates did not correlate with nitrate concentrations, and were thus expressed as a mean value for each treatment (Fig. 3). At 15°C, bioturbation stimulated nitrification rate by a factor of 1.8 compared with cores without larvae (ANOVA, $p < 0.05$), whereas at 10°C no enhancement of nitrification by chironomids was observed ($p > 0.05$). The average nitrification rates in control cores were not significantly higher at 15 than at 10°C ($p > 0.05$). The release of N_2O was found to be positively correlated to the rate of nitrification (Fig. 4; simple linear regression, $p < 0.01$).

Oxygen concentration in the water overlying the sediment was never below 70% of saturation during the incubations and, thus, oxygen was not expected to limit nitrification.

Denitrification

In contrast to N_2O emission and nitrification, total denitrification and denitrification of nitrate from the overlying water (d_w) was positively correlated to the concentration of water nitrate in both bioturbated and non-bioturbated cores and at both temperatures (Figs. 5 & 6; $p < 0.01$ for all treatments, simple linear regression). However, at both temperatures, total denitrification was more stimulated by nitrate in bioturbated cores than in non-bioturbated ones ($p < 0.01$, Student's *t*-test, comparing simple linear regression equations; Zar 1974). In addition, the stimulating effect was in all cases most conspicuous at 15°C ($p < 0.01$, Student's *t*-test, comparing simple linear regression equations). Bioturbation stimulation factors calculated at 10 and 15°C, as estimated from the regression slopes of total denitrification (d_{tot}) versus nitrate concentration, were 3.5 and 4.3, respectively.

Denitrification of nitrate from nitrification (d_n), i.e. coupled nitrification-denitrification, was found to be independent of the nitrate concentration in the overlying water whereas d_w was shown to be highly correlated to nitrate concentration (Figs. 5 & 6). The failure of d_n to correlate with nitrate concentration implies that the major constraints of the nitrogen isotope pairing method were met, i.e. that uniform mixing between the added $^{15}NO_3$ and the naturally occurring

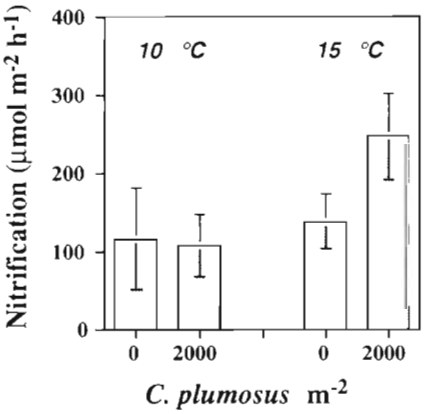

Fig. 3. Average rates of nitrification in eutrophic sediment from Lake Sövdesjön at 10 and 15°C in the presence and absence of *Chironomus plumosus* (2000 ind. m^{-2}). Mean ± SD, n = 20 to 24

$^{14}NO_3$ took place according to Nielsen (1992). d_n, calculated as an average over the nitrate gradient, was found to be highest in the presence of chironomids at both 10 and 15°C relative to cores without chironomids. d_n was measured as 44 ± 8 and 25 ± 6 µmol N m^{-2} h^{-1} at 10°C and 83 ± 20 and 30 ± 8 µmol N m^{-2} h^{-1} at 15°C in bioturbated and non-bioturbated cores, respectively. The bioturbation stimulation factor of d_n was 1.8 at 10 and 2.8 at 15°C.

The data for total denitrification (d_{tot}) shown in Figs. 5 & 6 were evaluated by multiple regression resulting in a 98% degree of explanation of the variation observed, expressed as µmol N m^{-2} h^{-1} by the following equation:

$$\log(d_{tot}) = 0.589\log(C_w) + 0.089\,B_{cp} + 0.056\,T_w - 0.0349$$

where C_w is the concentration in µM of nitrate in the overlying water, B_{cp} is the biomass of *Chironomus plumosus* larvae in g dry weight (DW) m^{-2}, and T_w is the temperature in°C of the overlying water. The equation demonstrates the linear increase in denitrification between 10 and 15°C, and between 0 and 5.8 g DW m^{-2} of chironomid biomass.

The molar ratio between produced N_2O and total denitrification, expressed as a percentage, decreased with increasing nitrate concentration in the overlying water in both bioturbated and non-bioturbated cores at both temperatures (Fig. 7). The relationship was expressed by simple logarithmic functions. In bioturbated cores the N_2O:N_2 ratio was slightly higher at 15 than at 10°C. The maximum ratio, 0.41%, was found at the lowest nitrate concentration in non-bioturbated cores at 15°C. The molar percentage of N_2O to denitrification (N_2) was always lower in bioturbated cores than in corresponding controls at both 10 and 15°C.

DISCUSSION

Emission of N_2O

Increased production and emission of N_2O from aquatic sediments is considered mainly as an effect of eutrophication, due to an increased load of nitrogen compounds (Samuelsson & Klemedtsson 1991). Emissions from marine environments have been estimated in Swedish coastal areas, from 44 kg N_2O-N km^{-2} yr^{-1} (Seitzinger 1988) to 63 kg N km^{-2} yr^{-1} (Samuelsson & Klemedtsson 1991) and from the Baltic Sea to ca 82 to 173 N km^{-2} yr^{-1} (Robertson 1991). From Swedish and Danish freshwater ecosystems the range of N_2O emission has been estimated at 5 to 500 kg N km^{-2} yr^{-1} (Robertson 1991). In the present study, N_2O emission from a lake sediment was calculated to amount to between 30 and 110 kg N_2O-N km^{-2} yr^{-1}, which is within the range of marine and freshwater ecosystems reported in the literature. The predominant epilimnion water temperature over the year in southern Swedish shallow lakes is close to 10°C. This implies that the data on N_2O emission for 10°C appear to be the most relevant figures when calculating an annual mean of emission from the eutrophic sediment, i.e. 30 and 37 kg N_2O-N km^{-2} yr^{-1} for the non-bioturbated and bioturbated sediment, respectively.

The ratio of released N_2O:N_2 from the sediment did not exceed 0.5% in any treatment, which corresponds to the finding that N_2O emissions seldom exceed 1% of N_2 emissions in marine areas, rivers and lakes (Seitzinger 1990).

However, it must be taken into account that the present data originate from a strictly laboratory set-up with constant environmental factors, i.e. high oxygen concentration in the water column, lack of newly settled planktonic material as well as absence of wind and wave induced resuspension of the sediment and advective pore water transport. This makes extrapolations to *in situ* conditions somewhat hazardous. Sedimentation of fresh organic material might periodically lower the oxygen pressure in the water close to the sediment surface and increase the emission of N_2O (Seitzinger et al. 1984, Seitzinger 1990). The opposite might be the result of increased advective pore water transport by wind and wave action, i.e. lower sediment N_2O emission due to enhanced movement of N_2O and other substances across the sediment-water interface as well as within the sediment which will facilitate the further reduction of N_2O.

Emissions of N_2O from aquatic sediments have been attributed to both nitrification and denitrification. In the present experiments, nitrous oxide emissions from the eutrophic sediment were not positively correlated with nitrate concentration in the overlying water, but were more or less constant over the nitrate gradient for each treatment. Other studies show increased N_2O emission from marine and estuarine sediment after

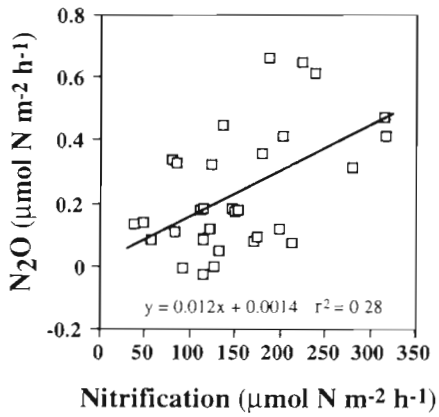

Fig. 4. Emission of nitrous oxide in relation to rates of nitrification in sediment from Lake Sövdesjön. Observe that the regression includes 4 treatments, 10 and 15°C, and bioturbated and non-bioturbated. The fitted line is based on simple linear regression ($p < 0.05$, n = 32)

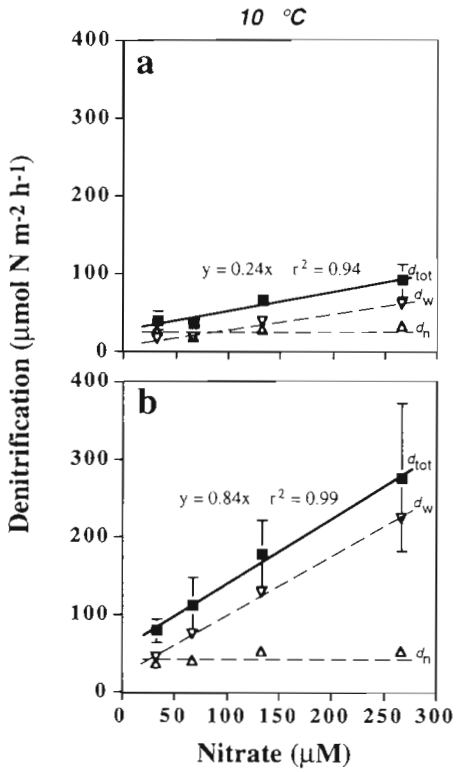

Fig. 5. Denitrification rates of nitrate from the water column (d_w), of coupled nitrification-denitrification (d_n) and total denitrification (d_{tot}) in sediment from Lake Sövdesjön at 10°C, in relation to nitrate concentration in (a) the absence and (b) the presence of *Chironomus plumosus* (2000 ind. m^{-2}). The fitted lines for d_{tot} are based on simple linear regression. n = 10 to 12

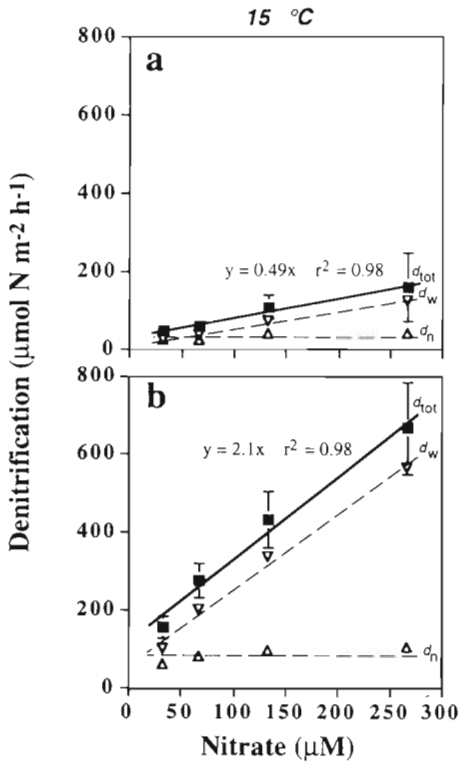

Fig. 6. Denitrification rates of nitrate from the water column (d_w), of coupled nitrification-denitrification (d_n) and total denitrification (d_{tot}) in sediment from Lake Sövdesjön at 15°C, in relation to nitrate concentration in (a) the absence and (b) the presence of *Chironomus plumosus* (2000 ind. m^{-2}). The fitted lines for d_{tot} are based on simple linear regression. n = 10 to 12

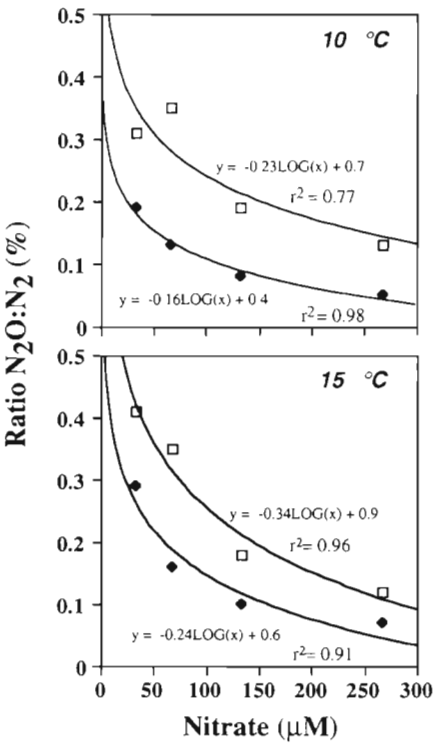

Fig. 7. Ratios of N_2O:N_2 released from the sediment of Lake Sövdesjön at 10 and 15°C, in relation to nitrate concentration in the presence (◆) and the absence (□) of *Chironomus plumosus* (2000 ind. m^{-2})

increased nutrient load (Nixon et al. 1984 in Seitzinger et al. 1988, Seitzinger et al. 1984, Seitzinger & Nixon 1985, Seitzinger 1990, Law et al. 1991). In one of these papers (Nixon et al. 1984 in Seitzinger et al. 1988), the increased N load caused an overall increase in total system production, benthic oxygen uptake and denitrification, but above all, a marked increase in the N_2O:N_2 ratio from 0.2 to 5.8%. The reason for this increase was attributed to denitrification, but was not further investigated by Nixon et al. (1984 in Seitzinger et al. 1988). A fundamental difference between this study and those of Nixon et al. (1984 in Seitzinger et al. 1988), Seitzinger et al. (1984) and Seitzinger & Nixon (1985) was that inorganic nitrogen was added as ammonium in their experiments which were performed in mesocosms with benthic-pelagic coupling. One explanation for the enhanced N_2O:N_2 ratio might be that the increased N load stimulated planktonic primary production, which in turn elevated sedimentation of organic material at the sediment surface. Elevated sedimentation can (if organic) affect O_2 consumption and H_2S production, which is known to inhibit denitrification in estuarine sediments, causing an insufficient reduction of N_2O to N_2 (Sørensen et al. 1980). Since marine and estuarine sediments accumulate more H_2S than lake sediments, H_2S was not expected to be of major significance in the present study. The investigated lake sediment was also continuously flushed with well-oxygenated water and the flow was adjusted to avoid oxygen concentration below 70% of saturation in the overlying water. These experimental conditions most probably prevented any production of H_2S in the upper part of the sediment and no signs of H_2S production were observed. In contrast to what was found by Nixon et al. (1984 in Seitzinger et al. 1988), the ratio of N_2O:N_2 in this study decreased with increasing load of nitrate, in both bioturbated and non-bioturbated cores at both temperatures (Fig. 7). The decreasing N_2O:N_2 ratio is explained by increasing emission of N_2 (denitrification) with increasing nitrate concentrations, whereas N_2O, despite the increased N load, was maintained at a constant level. Since ammonium was held at a constant and low level throughout the experiment (14 µmol N l^{-1}) and nitrification was stable over all nitrate concentrations, this might have resulted in a low and constant flux of N_2O. The nitrification rate was generally independent of nitrate concentration. Hence, my results corroborate the hypothesis that nitrification is the major source of N_2O. This hypothesis

is also supported by the coupling between emission of N_2O and nitrification observed in this experiment (Fig. 4). From the results of Elkins et al. (1978), it was demonstrated that nitrification may represent a dominant source of N_2O in both freshwater and marine ecosystems. The authors suggested that approximately 0.14% of the oxidised NH_4 ends up as N_2O. As a fraction of nitrification, N_2O calculated from the present study ranged between 0.09 and 0.22% over the different treatments, which agrees with values observed by Elkins et al. (1978).

Ratio of N_2O to N_2

Bioturbation by *Chironomus plumosus* larvae had a considerable stimulating effect on denitrification in the eutrophic sediment tested, as also reported by Svensson & Leonardson (1996) and Svensson (1997). The influence was most conspicuous at 15°C, at which temperature denitrification was increased 4.3-fold over larvae-free cores relative to 3.5-fold at 10°C. Bioturbation also increased N_2O emission to the overlying water, following the trend observed for nitrification. However, the N_2O:N_2 emission ratio was always lower in bioturbated cores compared to corresponding controls at the same temperature. An explanation might be found in the way larvae rework the sediment. Through burrowing activities, *C. plumosus* larvae increase the surface of the sediment-water interface (cf. polychaetes; Kristensen 1984). Invertebrates like *C. plumosus* are filter feeders and induce water circulation through their burrow by ventilatory pumping to collect food particles, as well as to obtain oxygen. An additional effect of this ventilation activity is the influx to the burrows of other electron acceptors besides oxygen, such as nitrate, and the simultaneous removal of reduced and inhibiting metabolites, such as sulphide and ammonium (Andersen & Kristensen 1991). The burrows of *C. plumosus* larvae provide favorable conditions for bacteria in the microenvironment associated with the tube walls (van de Bund et al. 1994). Thus, the larvae provide a more tight coupling between nitrification-produced N_2O and denitrification, which facilitates the subsequent reduction of N_2O to N_2. The oxic layer at the sediment-water interface is considerably thicker than it is in the burrow walls (Jørgensen & Revsbech 1985). This means that diffusion of nitrate from the burrow water to denitrifiers in anoxic sites under temporal or permanent anoxia within parts of the burrows is facilitated, as suggested by Pelegrí et al. (1994), whereby denitrification is stimulated. Subsequently, N_2O released in the upper part of the burrow will be transported by the *C. plumosus* induced ventilation current further down into the burrow.

Ventilating water through the burrows is not a continuous pattern. Mainly associated with the oxygen conditions in the overlying water, the larvae ventilate with a certain periodicity (Lindroth 1942, Leuchs 1986, Heinis & Crommentuijn 1992). During periods when the larvae do not irrigate, the residence time of the water inside the burrow is prolonged, whereby N_2O trapped in the burrows side can diffuse to denitrifiers in anoxic microzones. The coupling between the reduction of nitrate (nitrite) to N_2O and the step where N_2O is further reduced to N_2 is facilitated under these circumstances. The closer coupling between nitrification and denitrification was also supported in the present experiment when comparing d_n to total nitrification. In cores with chironomids, the coupling was 42 and 34% at 10 and 15°C, respectively, whereas in non-bioturbated cores this coupling never exceeded 22% at both temperatures.

Denitrification

The temperature dependence of denitrification in aquatic environments is, according to Seitzinger (1988), not that pronounced and is not easily distinguished from the influence of other factors since processes like nitrification and oxygen consumption also usually increase with temperature. Generally, denitrification is found to increase with increased temperature. In the present study, values of Q_{10} were calculated to be 2.0 in the non-bioturbated sediment and 2.5 in the sediment bioturbated by *Chironomus plumosus*. These values, calculated from the linear slopes of the regressions in Figs. 5 & 6, are within the range of values reported earlier in the literature for various aquatic sediments (Seitzinger 1988). The stimulating effect of bioturbation on denitrification is evidently more pronounced at higher temperatures. This effect is most certainly caused by the increased oxygen demand of the chironomid larvae, which are forced to ventilate more water through their burrows to compensate for the lowered partial pressure of oxygen, and the increased microbial oxygen consumption in the sediment at higher temperature (Lindroth 1942, Leuchs 1986). This was also supported in this study by the higher rate of O_2 consumption in bioturbated cores at 15°C relative to the consumption measured at 10°C. Enhanced ventilation activity will not only increase the availability of oxygen for animal respiration, but should also mobilise nitrate from the water phase to deeper sediment layers and result in increased sediment denitrification.

Since the present study was performed under aerobic conditions with sufficient pools of nitrate in the overlying water and with no additions of carbon, it can

be concluded that dissimilatory nitrate reduction to ammonium (DNRA) is of small or no importance relative to denitrification in the sediment of Lake Sövdesjön. DNRA is thought to be most significant at low nitrate and high organic carbon concentrations producing anaerobic conditions in the bottom water (Sørensen 1987, Cole 1988). The work of Kaspar & Tiedje (1981), which demonstrated that only about 10% of nitrate added to a eutrophic sediment was ammonified by this pathway supports this statement. It was also shown by the work of Binnerup et al. (1992) that DNRA was also very low (0 to 20%) when ^{15}N-nitrate was added to a bioturbated estuarine sediment.

Nitrification

Bioturbating tube-constructing benthic animals are normally found to enhance denitrification to a greater extent than nitrification (Kristensen et al. 1991, Pelegrí & Blackburn 1995a). Nitrification should only respond in relation to the sediment surface extension as it is supported mainly by ammonium produced within the sediment, whereas denitrification is also supported by the animal's active downward transport of nitrate to anaerobic zones in addition to the nitrate formed in the nitrification process. Further, the water pumping activity of the chironomids mediates a coupling between nitrification and denitrification by transporting the produced nitrate alongside the burrow (as discussed for N_2O). The coupling between nitrification and denitrification might, in this way, be close to 100% (Pelegrí et al. 1994). Half of the nitrate produced in non-bioturbated sediment is considered to diffuse up into the overlying water, and the other half to diffuse down to sites for denitrification (Blackburn & Henriksen 1983, Jensen et al. 1993). It has been shown here that denitrification was stimulated by the chironomids to a greater extent than nitrification. At 15°C denitrification and nitrification were enhanced 4.3- and 1.8-fold, respectively. The enhancement at 10°C was 3.5 and 1.0 for the 2 processes, respectively. These figures are in accordance with the expected higher stimulation of denitrification compared with nitrification (see above). The lack of stimulation of nitrification by the chironomids at 10°C is not easily explained (1.3-fold, not significant). However, since the enhancement is obvious at 15°C, this lack of stimulation might be the result of a deactivated flora of nitrifiers that needs higher temperature to become triggered. Temperature might only play a regulatory role for the nitrification activity in sediments where oxygen penetration and ammonium availability are more important controlling factors (Hansen et al. 1981). Higher temperatures lower the depth of oxygen penetration and depress nitrification activity. This could explain why there was no stimulation of nitrification when temperature was raised from 10 to 15°C in the non-bioturbated sediments. Oxygen penetration depth was not measured, but was presumably lower at the higher temperature as also indicated by the lower O_2 consumption rate at 10°C. The rate of ammonium production is likely higher in the anoxic part of the sediment at 15°C relative to the rate at 10°C (Blackburn 1980). This may explain why the stimulation of nitrification by the chironomids was lower at 10°C than at 15°C in this study; however, this cannot be confirmed from the present data.

Conclusion

The present study demonstrates that tube-dwelling larvae of *Chironomus plumosus* can have a strong influence on inorganic nitrogen turnover in eutrophic lake sediments, by increasing the sediment release of N_2O and the rates of nitrification and denitrification. However, even though the chironomids increased N_2O emission (suggested to originate from nitrification), the flux of N_2O was always less than 1% of the N_2 production, as generally observed in natural aquatic sediment. This indicats that bioturbated eutrophic sediment does not contribute more N_2O to the atmosphere than non-bioturbated sediment. The increase in N_2O emission by the chironomids is highly over-shadowed by the strong positive influence on denitrification by the same larvae, indicating that bioturbation can be an important contributor to overall nitrogen retention in lakes, ponds and other wetlands.

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