Vol. 12: 275–284, 1997 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published May 29

Denitrification and microphytobenthic NO_3^- consumption in a Danish lowland stream: diurnal and seasonal variation

Anette Pind, Nils Risgaard-Petersen*, Niels Peter Revsbech

Department of Microbial Ecology, Institute of Biological Sciences, Bd. 540, University of Aarhus, DK-8000 Aarhus C, Denmark

ABSTRACT: Seasonal and diurnal variation of denitrifying activity was determined in a microphyte-covered lowland stream sediment using an isotope pairing technique based on the stable ^{15}N-isotope. By this approach it was possible to divide total denitrification (D_{tot}) into denitrification based on NO_3^- diffusing from the overlying water (D_w) and coupled nitrification-denitrification within the sediment (D_n). D_{tot} exhibited seasonal variations between 0 and 17 mmol m^{-2} d^{-1} with a pronounced minimum during winter. D_w showed the same seasonal pattern as D_{tot} and accounted for 75 to 90% of the total denitrification activity. In contrast to the pattern previously found in estuarine systems, a minimum in D_w activity was found during the winter period when the NO_3^- concentration in the water was highest. This was apparently due to a dramatic increase in water discharge during the winter period increasing the erosion of the stream bed, thereby reducing the carbon availability for the denitrifiers and probably also their numbers. Temporary sediment N accumulation was estimated as the difference between rates of total net dissolved inorganic nitrogen uptake (NH_4^+, NO_3^-) and total denitrification ($D_n + D_w$). The rate of temporary sediment N accumulation varied seasonally between 0 and 13 mmol N m^{-2} d^{-1} with a pronounced maximum during spring (April–May) coincident with the microphytobentic spring bloom. Most of the N load to the stream occurred during the winter and as a consequence the retention capacity of the system was low on an annual basis. Denitrification and accumulation in the stream were both found to account for less than 1% of the N transported on an annual basis, while approximately 60% was removed by denitrification and sediment accumulation during the summer period (May–August).

KEY WORDS: Denitrification · N accumulation · N load

INTRODUCTION

Rivers and streams that run through urban and agricultural areas receive large inputs of nitrogen and phosphorus, of which especially antropogenic inputs of nitrate are of environmental concern (Hill 1983). In contrast to most marine environments, where nitrogen is the major controlling factor for primary production (Ryther & Dunstan 1971), the lowland fresh waters often contain dissolved inorganic nitrogen (DIN; NO_3^-, NH_4^+) in abundance, and riverine inputs are therefore significant contributors to eutrophication in the coastal marine areas.

The retention of nitrogen in river systems may be investigated by mass balance studies (Hill 1983), and thereby the permanent and temporal retention caused by denitrification, in-stream accumulation (Svendsen & Kronvang 1993) and assimilation by primary producers can be measured (Cooper & Cooke 1984). Sedimentation and incorporation into biomass is likely to retain the nitrogen for periods ranging from weeks to months, and the temporal retention is subject to a pronounced seasonal variation (Svendsen & Kronvang 1993). Denitrification, the bacterial reduction of NO_3^- to N_2 gas, is the only significant pathway by which inorganic nitrogen is lost from ecosystems.

Denitrification activity is regulated by the population size, the availability of organic carbon as energy source, and the oxidant (NO_3^-) supplied by diffusion from the overlying water column or by nitrification in

*Addressee for correspondence. Present address: National Environmental Research Institution, Vejsøvej 25, DK-8600 Silkeborg, Denmark. E-mail: nri@dmu.dk

the sediment. Since denitrification is an anaerobic process, and thus probably restricted to the anoxic sediment, the diffusion of NO_3^- and thereby the denitrification activity is regulated by the oxygen penetration into the sediment (Nielsen et al. 1990). The penetration of O_2 into the sediment is directly linked to O_2 respiration and production in the upper sediment strata, and therefore denitrification may be regulated by these processes (Christensen et al. 1990). For microphyte-covered sediments, seasonal as well as diurnal variation are found for O_2 consumption/production and oxygen penetration. Similar variations are therefore found for denitrification (Christensen et al. 1990).

As a consequence of the water current in streams, the diffusion of NO_3^- into the sediment may be supplemented by convective transport, which can thereby stimulate denitrification activity (Cooke & White 1987). However, an increased discharge will cause disturbance and erosion of the stream bottom and thus reduce the availability of readily mineralizable organic carbon and the numbers of denitrifiers in the sediment (Cooper & Cooke 1984, Hill 1988).

In this study the isotope pairing technique described by Nielsen (1992) was applied to stream sediment kept in microcosms under *in situ* conditions of light, temperature and concentrations of DIN By only analyzing freshly collected sediment, it was possible to investigate both seasonal and diurnal variations in denitrification based on NO_3^- diffusing from the overlying water (D_w) and denitrification coupled to nitrification in the sediment (D_n) as well as fluxes of inorganic nitrogen and oxygen across the sediment-water interface. Based on these measurements, the nitrate retention by accumulation in algal biomass was estimated.

MATERIALS AND METHODS

Study area and sampling. The Gelbæk is a first order lowland stream located in the eastern part of Jutland, Denmark, and is primarily supplied by drainage water from surrounding agricultural fields. Water discharge is thus connected to rainfall, which explains the marked differences in summer and winter discharge. From early spring (March–April) a benthic community of microphytes develops at the sediment surface, and remains abundant throughout summer and autumn. Diatoms (*Navicula* sp.) were the dominant primary producers during an algal bloom in April and May, but the diatom population decreased during summer and fall when the stream was shaded by herbs and Red Alder trees (*Alnus glutinosa*) growing along the bank. Tube-forming chironomid larvae appeared in abundance in the sediment during early summer (June). A further description of the stream is presented by Christensen & Sørensen (1988) and Svendsen & Kronvang (1993).

A small reach with moderate flow velocities and sandy sediment was chosen for sampling. Sediment was sampled, with intervals of 4 to 6 wk, from late spring 1993 until early summer 1994. At each sampling date 20 intact sediment cores (10 in 36 mm and 10 in 26 mm inner diameter Plexiglas tubes) were sampled randomly by hand. *In situ* water temperature and light intensity (Li-192Sa, Li-Cor, Lincoln, NE, USA) at the sediment surface were measured on each sampling date. Data concerning discharge and NO_3^--load to the stream were supplied by L. Svendsen, NERI, Silkeborg, Denmark.

Incubation procedure. On return to the laboratory the 36 mm cores were adjusted to a sediment depth of 5 cm and a water column depth of 15 cm. The overlying water in the cores was stirred by a small Teflon-coated magnet positioned 10 cm above the sediment surface. The small magnet received momentum from an external rotating magnet (100 rpm). The core tubes were left uncapped overnight in an open, dark reservoir. The water in the reservoir was a 2:1 mixture of water from the location, filtered through a Whatman CF/F filter and artificial freshwater (AFW; 0.18 mg $Na_2HPO_4 \cdot 2H_2O$, 20 mg $CaCl_2 \cdot 2H_2O$, 20.5 mg $MgSO_4 \cdot 7H_2O$, and 0.21 mg $NaHCO_3$ l^{-1}) with *in situ* concentrations of inorganic nitrogen. The temperature of the water was held at *in situ* conditions and O_2 concentration was held at air saturation. For the 7 h prior to incubation, 5 cores were preincubated in the dark and 5 were exposed to *in situ* light intensities provided by a 400 W mercury lamp. The preincubation of the cores ensured steady state conditions of oxygen profiles during the denitrification/flux measurements.

Before the start of the incubations, the core tubes were transferred to a water bath with identical conditions in terms of temperature, light, and concentrations of inorganic nitrogen and O_2 as in the preincubation bath. However, in the incubation bath the NO_3^- added to AFW was ^{15}N-labeled and the resulting ^{15}N enrichment of the NO_3^- pool was thus ~30%. This 1:2 dilution of the *in situ* water with ^{15}N-enriched AFW allowed us to maintain overall NO_3^- concentrations at *in situ* levels, and NO_3^- consuming processes (e.g. assimilation and denitrification) therefore were not artificially affected through addition of $^{15}NO_3^-$. The water in the reservoir was allowed to equilibrate with sediment pore water for ~20 min before the assay was started.

Incubations were initiated by closing the core tubes with a rubber stopper for dark incubations or a transparent Plexiglas lid for light incubations. The duration of the incubation varied between 1 and 8 h, ensuring

that the change in O_2 concentration never exceeded 20% of the initial O_2 concentration.

Water samples for ^{15}N-N_2 analysis and for determination of inorganic nitrogen (NO_3^-, NO_2^- and NH_4^+) and O_2 concentrations were collected immediately before closing the core tubes, and then again immediately after the incubations were terminated by removing the rubber stoppers or lids. Samples for ^{15}N-N_2 analysis were collected with a glass syringe, transferred to 6 ml glass vials (Exetainers, Labco, High Wycombe, UK), and preserved with 0.1 ml 7M $ZnCl_2$ solution. Samples for O_2 determination were analyzed by Winkler titration within a few hours after sampling, and samples for determination of NO_3^-, $^{15}NO_3^-$, and NH_4^+ were frozen (–18°C) for later analysis. After collection of the necessary water samples from a core, the remaining water column except for approximately 1 cm was removed, and 0.5 ml of the $ZnCl_2$ solution was added to the core. The pore water in the 5 cm deep sediment core and the remaining water column were carefully mixed with a glass rod and a 6 ml sample of the resultant slurry was collected with a syringe, transferred to a glass vial (Exetainer) and preserved with 0.2 ml 7M $ZnCl_2$.

The chlorophyll *a* (chl *a*) content (corrected for pheopigments) in the upper 0.5 cm of the sediment was determined on separate 26 mm sediment cores (n = 4). The chl *a* content was determined by ethanol extraction for 24 h followed by spectrophotometric analyses at 665 nm (Wintermanns & De Mots 1965). Sediment porosity was determined at each sampling date on 4 separate 26 mm cores with the same sediment depth as the cores used for incubations.

Analytical procedures. NH_4^+ was analyzed colorimetrically by the salicylate hypochlorite method (Bower & Holm-Hansen 1980). Concentrations of NO_3^- + NO_2^- were determined on a flow injection analyzer (Tecator, Högenäs, Sweden) by the method described by Grashoff et al. (1983). The $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N_2 was analyzed by mass spectrometry. The N_2 gas dissolved in the water and slurry samples was extracted into an added 10% (vol/vol) helium-headspace by vigorously shaking for 5 min. By use of a gastight glass syringe, gas from the helium headspace was injected into an isotope ratio mass spectrometer (Sira Series II, V.G. Isotech, Middelwich, UK) in line with a gas chromatograph, and the concentrations of the ^{15}N-N_2 isotope species ($^{14}N^{15}N$ and $^{15}N^{15}N$) were analyzed. The ^{15}N abundance in NO_3^- (at.% NO_3^-) was determined by mass spectrometry after bacterial reduction of the NO_3^- to N_2 by use of a pure culture of *Pseudomonas aeruginosa*, as described by Risgaard-Petersen et al. (1993).

Calculations. The production rate (in µmol m^{-2} h^{-1}) of both ^{15}N-labeled N_2 species ($p^{15}N_2$) was calculated as follows:

$$p^{15}N_2 = \frac{[(C_{water} - C_{ini}) \times (vol_1 - vol_3) + (C_{slurry} - C_{ini}) \times (vol_2 \cdot \phi + vol_3)]}{\Delta t} \times \frac{1}{A}$$

where C_{water} and C_{slurry} are the concentrations of the isotope in the water and slurry respectively, C_{ini} is the initial concentration of the isotope in the water, vol_1 is the volume of the water column, vol_2 is the volume of the sediment, vol_3 is the volume of the water column after sampling but before mixing, ϕ is the sediment porosity, A is the surface area of the sediment surface, and t is the incubation time.

Denitrification rates D_{14} and D_{15} based on $^{15}NO_3^-$ and $^{14}NO_3^-$ respectively were estimated from the production of ^{15}N isotopes (Nielse 1992):

$$D_{15} = p(^{15}N^{14}N) + 2 \times p(^{15}N^{15}N)$$

and

$$D_{14} = \frac{p(^{15}N^{14}N)}{2 \times p(^{15}N^{15}N)} \times D_{15}$$

where $p(^{15}N^{14}N)$ and $p(^{15}N^{15}N)$ are the rates of production of the 2 dinitrogen species ($^{15}N^{14}N$ and $^{15}N^{15}N$).

The rate of denitrification of nitrate diffusing from the water column (D_w) was calculated from D_{15} and the ^{15}N abundance of nitrate in the water column:

$$D_w = \frac{100}{\text{at.\% } NO_3} \times D_{15}$$

where at.% NO_3 is the ^{15}N at.% of NO_3^- in the water column calculated as described by Risgaard-Petersen et. al. (1993). The rate of coupled nitrification-denitrification (D_n) was finally calculated as the total denitrification ($D_{14} + D_{15}$) minus D_w.

Diurnal flux or denitrification rate (R_d) was calculated as follows:

$$R_d = r_{light} \times \text{day length} + r_{dark} \times (24 - \text{day length})$$

where r_{light} and r_{dark} are the fluxes or denitrification rates that were measured in light and dark incubated cores respectively. Day length was defined as the time from sunrise to sunset.

RESULTS

Discharge and N load

Maximum discharge was observed during the winter months, and in both January and March sudden floods occurred in connection with episodes of snow melt. Summer base flow rarely exceeded 20 l s^{-1}, whereas the discharge was >100 l s^{-1} throughout the winter months and frequently reached 500 l s^{-1} (Fig. 1A). The N load per unit area of creek sediment in the Gelbæk was 36 t

N ha^{-1} yr^{-1}. However, great seasonal variation in the N load was observed. During the winter months (December–January) N loading was maximal and amounted to ~11 t N ha^{-1} mo^{-1}. During the summer months (May–August) the load was <100 kg N ha^{-1} mo^{-1}.

DIN concentrations

The nitrate concentration was low during summer with a minimum in July 1993, which coincided with a very low water flow. During autumn the NO_3^- concentration rose and remained above 500 µM throughout winter. From late April the NO_3^- concentration decreased again and reached a low summer concentration in June (Fig. 1B). Since the major input of water to the Gelbæk is subsurface drainage from agricultural catchment, both the total water discharge and the NO_3^- concentration in the stream are closely correlated with the amount of precipitation.

The concentration of NH_4^+ was relatively low throughout the seasons and with one exception never exceeded 50 µM. The high ammonium concentration in July 1993 coincided with low water flow and low NO_3^- concentration (Fig. 1B).

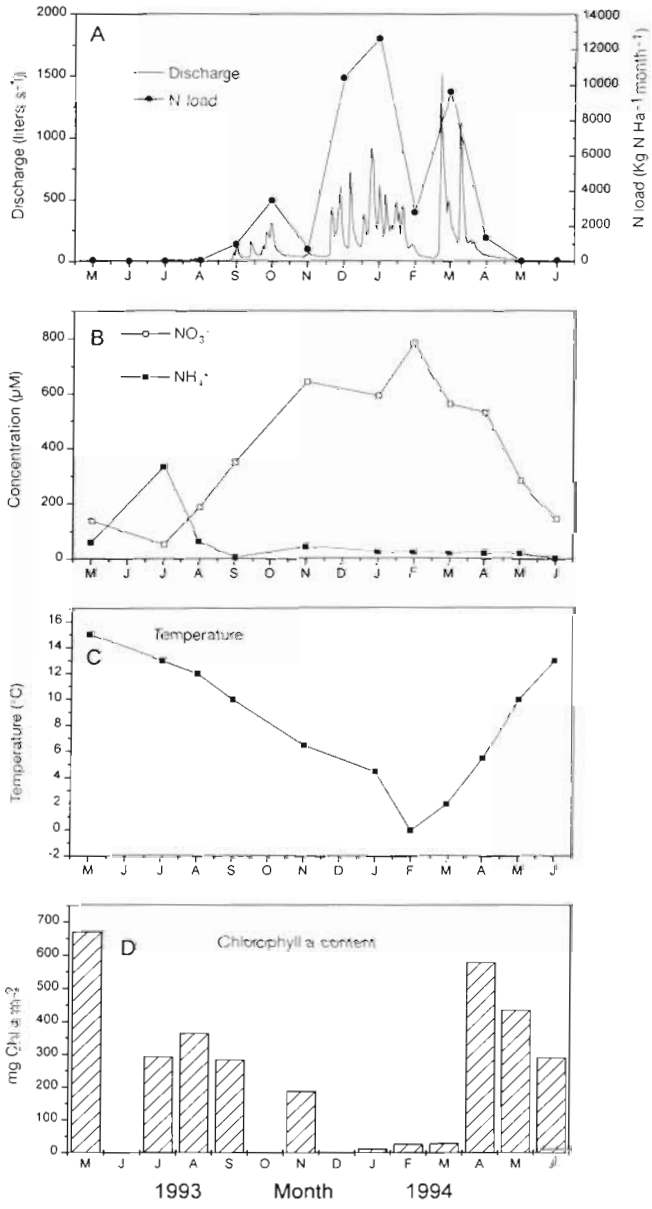

Fig. 1. Seasonal variation of (A) water discharge and N load to the Gelbæk, Denmark; (B) NO_3^- and NH_4^+ concentrations; (C) *in situ* temperature; and (D) chlorophyll *a* content of the upper 5 mm of sediment

Temperature

A pronounced seasonal variation was found in the temperature of the stream (Fig. 1C). Maximum of 13 to 15°C were observed in late spring and early summer, and the temperature then decreased gradually until an absolute minimum of 0°C was reached in February when the stream was partly covered by ice.

Chlorophyll content

The chl *a* content can be regarded as a measure of the biomass of microalgae, which constituted a source of readily mineralizable organic carbon in the sediment. Highest values were achieved during April-May, with a maximum of 600 to 700 mg m^{-2} occurring in May 1993. Thereafter the content decreased and remained at approximately 300 mg chl *a* m^{-2} throughout the summer (Fig. 1D). The reduction in algal biomass coincided with the foliation of the alder trees and shrubs along the stream bank, which limited the light availability for primary production. The chl *a* content decreased steadily during the autumn, and in the period January–March values of less than 50 mg chl *a* m^{-2} were found. This reflected low primary production caused by low light. Furthermore, there were at least 2 events of extreme discharges in March 1994 (Fig. 1A), in which the algal biomass could have been washed away together with other small-size sediment constituents.

Fluxes between the sediment and the water column

Oxygen

A pronounced maximum in photosynthetic activity, measured as net O_2 production, was observed in connection with the blooms of benthic diatoms in April

and May, 1994, and the primary production during this period far exceeded respiration with a resulting net O_2 efflux during illumination of 6 to 8 mmol O_2 m^{-2} h^{-1}. Occasionally, respiration in the sediment during illumination exceeded the photosynthetic O_2 production, but the net oxygen uptake during illumination was always less than the corresponding value in the dark (Fig. 2A).

A seasonal variation was also observed for sediment oxygen uptake in the dark. Throughout spring and summer the activity was around 2 mmol O_2 m^{-2} h^{-1}, but the respiration then decreased gradually during autumn to reach a minimum of 100 to 500 µmol O_2 m^{-2} h^{-1} during winter. The O_2 uptake remained low until late April when the activity increased and approached those observed during summer. In June 1994 a pronounced increase in respiration was observed which seemed to be caused by a large number of chironomid larvae inhabiting the sediment. This was reflected in the O_2 budget of the illuminated sediment, as the respiration in June 1994 markedly exceeded the photosynthetic activity, although there was a very high net oxygen production in the preceding month. The heterogeneous distribution of invertebrate activity was furthermore reflected in a pronounced variation in all the biological activities measured in June.

On a diurnal basis a net production of oxygen only occurred in November 1993 and April–May 1994 (Fig. 3A), in connection with high chl *a* contents and good light conditions at the sediment surface.

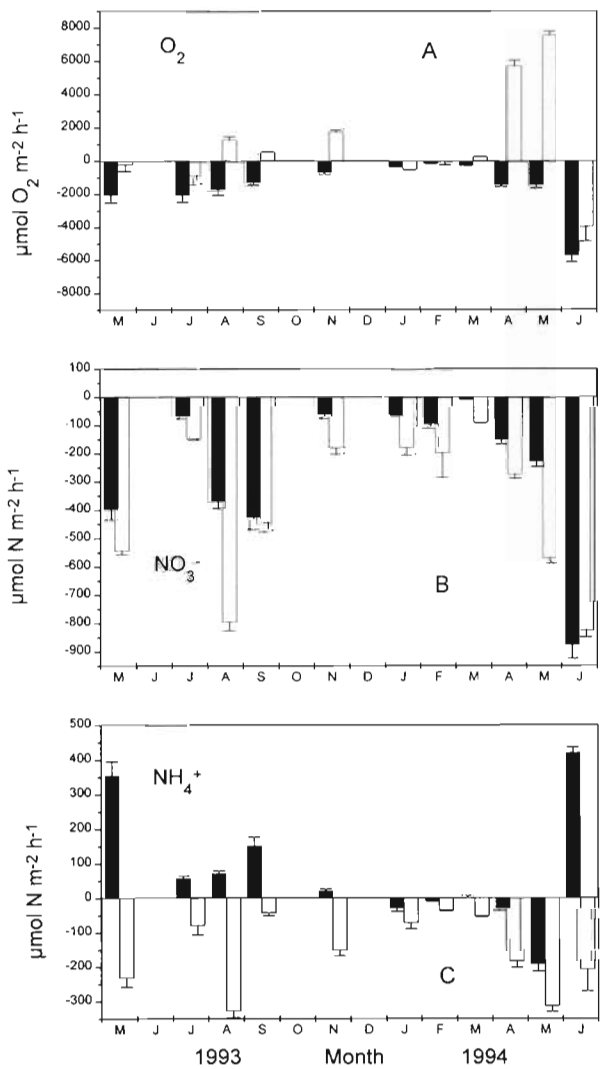

Fig. 2. Seasonal variation of (A) O_2, (B) NO_3^-, and (C) NH_4^+ fluxes between the sediment and the water column. Rates obtained in dark- and light-incubated cores are illustrated as solid and open bars respectively. Negative values represent fluxes directed from the water column toward the sediment. Error bars represent standard error of the mean (n = 5)

Dissolved inorganic nitrogen

Throughout the year, a net NO_3^- uptake by the sediment occurred both during illumination and during darkness (Fig. 2B). Generally, the NO_3^- uptake was stimulated by light (2-way ANOVA, $p < 0.01$). Nitrate uptake was always higher or equal (September 1993 and June 1994) in the light-incubated cores as compared to dark-incubated cores. A pronounced effect of light was observed in April, May, and August when the chl *a* values were highest. The NO_3^- uptake was highest during May–June and August–September (400 to 800 µmol N m^{-2} h^{-1}) while the uptake was below 200 µmol m^{-2} h^{-1} from November to March. The sediment NO_3^- consumption was thus lowest during the period with high concentrations of NO_3^- in the stream water. However, a low NO_3^- uptake in July 1993 also coincided with a low NO_3^- concentration in the overlying water.

There was pronounced diurnal variation in the NH_4^+ flux across the sediment-water interface, and light significantly stimulated the NH_4^+ uptake (2-way ANOVA, $p < 0.01$) (Fig. 2C). A net NH_4^+ uptake by the sediment always occurred during illumination while there usually was a net release in the dark. An exception occurred during the algal bloom in April and May, 1994, when sediment NH_4^+-uptake occurred in the dark, coincident with increased uptake during illumination and high primary production. A small uptake of NH_4^+ by the sediment during darkness was also observed during the winter months (January and February 1994). The considerable NH_4^+ release observed in June 1994 was coincident with a high density of infauna. In general, the fluxes of NH_4^+ across the sediment surface were highest from late spring until autumn, while the fluxes were low during winter, even approaching zero in the dark.

On a diurnal basis the total flux of DIN (NO_3^- and NH_4^+) was always directed into the sediment (Fig. 3B), and DIN was thus removed from the water column during the whole period, although a minimum with very low values of exchange was observed during the winter when the NO_3^- concentration in the stream water was highest (Fig. 1B).

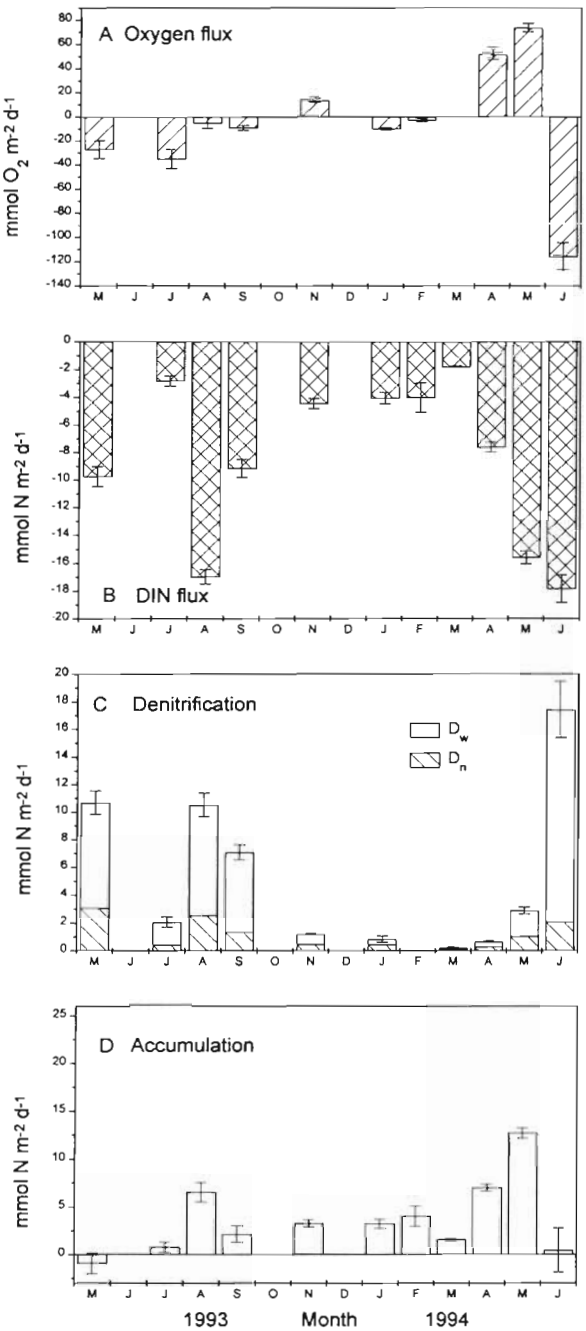

Fig. 3. Seasonal variation of (A) diurnal average O_2, (B) DIN fluxes, (C) rates of denitrification, (D) and estimated rates of in-stream N accumulation. Error bars represent standard error of the mean (n = 5)

Denitrification

There was a marked seasonal variation in the denitrification activity where the total denitrification activity (D_n + D_w) varied between 0 and 750 µmol m^{-2} h^{-1} (mean 200 µmol m^{-2} h^{-1}) (Fig. 4). During winter and early spring (November–April) there was virtually no denitrification coinciding with low O_2 respiration, suggesting overall low microbial activity during the period of high discharge. An absolute minimum in activity was observed in February when there was no measurable N_2 production. From late April the activity rose and reached a maximum in late May and in June. The pronounced drop in the beginning of July coincided with a drop in NO_3^- concentration.

Denitrification of NO_3^- diffusing from the overlying water (D_w) exhibited the same annual variation as total denitrification, with activities up to 650 µmol m^{-2} h^{-1} (Fig. 3A), and accounted for 75 to 90 % of the total denitrification activity during the season. The coupled nitrification-denitrification, (D_n) fluctuated less than D_w, but it showed the same pattern (Fig. 4B). D_n varied between 0 and 170 µmol m^{-2} h^{-1} and accounted for 10 to 25 % of total denitrification with maxima in May and August.

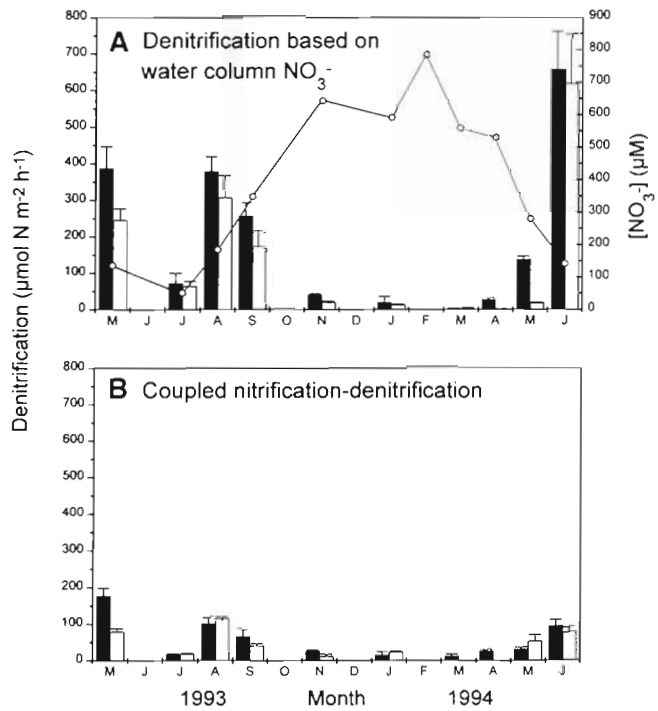

Fig. 4. Seasonal variation during illumination (open bars) and during darkness (solid bars) of (A) denitrification based on NO_3^- diffusing from the overlying water (D_w), and (B) coupled nitrification-denitrification (D_n). Error bars represent standard error of the mean (n = 5). (o) Nitrate concentrations in the water column

On a yearly base there was no general significant effect of light D_w (2-way ANOVA, $p > 0.1$). However, denitrification was significantly reduced in the light during November 1993 and April–May 1994 (2-way ANOVA, $p \ll 0.01$). During these months the sediment was net O_2 producing (Fig. 3A), and our finding may therefore suggest that only during these months were the benthic microalgae sufficiently active to affect the diurnal variation of denitrification activity.

D_n was reduced during illumination (2-way ANOVA, $p = 0.04$). On average D_n measured in the light-incubated cores corresponded to approximately 90% of the activity in the dark. In a few cases the activity in the illuminated cores exceeded the value of those in the dark. These occasions coincided with peaks in photosynthetic activity and high NH_4^+ concentrations in the overlying water in August 1993 and May 1994.

DISCUSSION

Seasonal variation of denitrification

In this study of the nitrogen cycle in stream sediment we used the isotope pairing technique to quantify denitrification. We found that denitrification reached maxima in May–June of 560 mol N m^{-2} h^{-1} in 1993 and of 750 mol N m^{-2} h^{-1} in 1994. In a previous study of denitrification in the Gelbæk in 1987 by Christensen et al. (1990), denitrification was quantified by means of the acetylene inhibition technique, and the authors found maximum activities up to 1400 mol N m^{-2} h^{-1} in June. However, in the study of Christensen et al. (1990), NO_3^- concentrations in the water column were about twice as high as NO_3^- concentrations during May and June in the present study. Since NO_3^- controls denitrification (Christensen et al. 1990) the discrepancy between our data and the data from this earlier denitrification study is probably due to a different NO_3^- load to the stream. Denitrification rates corrected for differences in NO_3^- concentration in the overlying water were in the same range for the 2 studies during the high productivity period from April to September.

In general, a good agreement between denitrification estimates by the acetylene inhibition technique and the isotope pairing technique can be expected for the Gelbæk, despite the fact that the acetylene technique, which inhibits nitrification, does not record coupled nitrification-denitrification (D_n). Our results indicate that D_n was always below 25% of the total denitrification activity; nitrification therefore does not contribute significantly as a NO_3^- source for denitrification.

It is possible, however, that we underestimated D_n during the winter incubations (January–February 1994), when the O_2 uptake rates were low (Fig 2A). The low O_2 consumption of the sediment implied a relative deep penetration of O_2 into the sediment (3 to 7 mm, as calculated from the equation of Revsbech et al. 1980), and it is possible that the $^{15}NO_3^-$ profile did not reach steady state before the incubation was initiated (i.e. 20 min after addition of the isotope). A steady-state $^{15}NO_3^-$ profile is a basic presumption for a correct estimation of D_n by the isotope pairing technique (Nielsen 1992). Simulations of $^{15}NO_3^-$ diffusion from the overlying water to the denitrification zone at the oxic-anoxic interface showed that it took about 1 to 4 h to reach steady state (data not shown). Our estimate of D_n is therefore conservative. However throughout the seasons D_n was always <9% of the O_2 consumption rate in the dark-incubated sediment. The overall low O_2 consumption rate of the sediment during January–February may therefore suggest that D_n was unimportant as a N-sink during these months.

Based on previous observations of proportionality between NO_3^- supply and denitrifying activity, NO_3^- availability has been proposed to be the major controlling factor for denitrification (Christensen & Sørensen 1988, Christensen et al. 1989, Nielsen et al. 1990). Also, in recent studies of the seasonal variation of denitrifying activity in Danish estuaries, a very close correlation was found between the availability of NO_3^- and the denitrifying activity throughout the season (Nielsen et al. 1995, Rysgaard et al. 1995). We have only found a similar correlation to NO_3^- availability in the Gelbæk for the summer months when D_w was regulated by the NO_3^- concentration. On an annual basis, however, no correlation was found, since an absolute minimum in D_w was observed during winter when the NO_3^- concentration in the stream water was high (Fig 3A). Thus, in the Gelbæk some other factors apparently cause reduced denitrifying activity during the period with most NO_3^- in the overlying water.

For streams at temperate latitudes an increase in NO_3^- concentration tends to be associated with an increase in stream discharge causing erosion of the stream bed (Hill 1988). The water current is thus likely to be the primary factor governing denitrification. Cooke & White (1987) found an increase in denitrification under moderate discharges compared to base flow conditions for a given availability of organic matter, probably caused by deeper O_2 and NO_3^- penetration under higher flow. However, increased discharges tend to cause erosion of the stream sediment, leading to a loss of particulate organic material from the sediment and consequently loss of substrates for microbial metabolism (Hill 1988). The very low activity of both denitrification and O_2 consumption during the winter months with high water discharge was apparently a consequence of such wash-out of organic substrate for

the heterotrophic community, and probably also a wash-out of the heterotrophic community itself.

Denitrification of NO_3^- supplied from the water column can be estimated from the model of Christensen et al. (1990). This model links the process of denitrification to sedimentary O_2 uptake and the concentration of NO_3^- in the water column. The basic assumption underlying the model is that D_w exclusively is controlled by diffusion of NO_3^- from the water column, and that NO_3^- assimilation and dissimilatory NO_3^- reduction to NH_4^+ is negligible. The model is expressed as follows:

$$D_w = F_{O_2} \times \alpha \times \left(\sqrt{\left(1 + \frac{D_{NO_3^-}}{D_{O_2}} \times \frac{C_{NO_3^-}}{C_{O_2}} \times \frac{1}{\alpha} \right)} - 1 \right)$$

where F_{O_2} is the sediment O_2 consumption rate, C_{O_2} and $C_{NO_3^-}$ are the respective concentrations, D_{O_2} and $D_{NO_3^-}$ are the respective diffusion coefficients, and α is the ratio between the volume-specific denitrification and O_2 respiration rates. Inserting measured values for sediment O_2 consumption during nighttime, NO_3^- and O_2 concentrations in the water column, and setting α = 0.8, and $D_{NO_3^-}/D_{O_2}$ = 0.82 (Christensen et al. 1989), resulted in the denitrification rates presented in Fig. 5.

In general, there was a good agreement between the seasonal variation in modeled and measured data, suggesting that denitrification was controlled mainly by diffusion. The very low rates observed during the winter months could therefore be explained by the low O_2 consumption rates. Low O_2 consumption implies a relative deep O_2 penetration depth, and consequently a long diffusional distance between the water column and the denitrification zone.

Very low measured D_w values as compared to the modeled denitrification rates were observed in November 1993 and April–May 1994. During these months

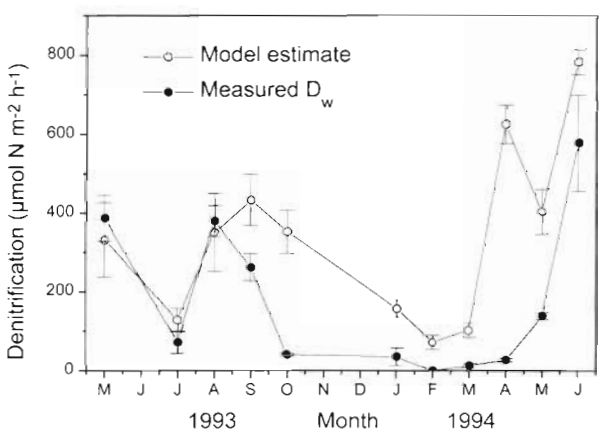

Fig. 5. Modelled vs measured denitrification rates in dark-incubated cores. Error bars represent standard error of the mean (n = 5)

the sediment was net O_2 producing (Fig 3A), and our finding may therefore suggest that the benthic microalgae during these months were sufficiently active to reduce the denitrification activity. Probably the NO_3^- uptake by the benthic algae reduced the NO_3^- supply to the denitrifiers.

On average, denitrification of NO_3^- diffusing from the overlying water accounted for ~3.78 mmol N m^{-2} d^{-1} (mean of all incubations; Fig. 3C) which equals 193 kg N ha^{-1} yr^{-1}. The annual N load to the stream was about 36 t N ha^{-1} (Fig. 1A). Thus only about 0.5% of the N transported was denitrified on an annual basis. However, a pronounced seasonal variation was observed, and with the high denitrifying activities during the summer (May, June and August), the denitrification could account for 40% of the N load.

Comparable low values for annual N removal have been found for the Danish estuary Nordsminde Fjord (Nielsen et al. 1995), where the removal accounted for 2 to 3% of the annual NO_3^- load.

Nitrogen accumulation in the stream

A common observation when working with sediments covered by benthic microphytes is a large difference between the gross NO_3^- consumption by the sediment and the denitrification of NO_3^- diffusing from the overlying water (Rysgaard et al. 1993, Risgaard-Petersen et al. 1994). Likewise, the total exchange of dissolved inorganic nitrogen (DIN) as NO_3^- and NH_4^+ across the sediment surface can be compared to the total denitrifying activity. When the total sediment uptake of DIN (Fig. 3B) was compared with the total release as N_2 gas due to denitrification (Fig. 3C), a surplus of N uptake was observed. This extra N uptake apparently represents N accumulation in the stream and the in-stream N accumulation (Fig. 3D) can thus be estimated as the difference between NO_3^- plus NH_4^+ flux (N_{input}) to the sediment and the total output of N_2 due to denitrification (N_{output})

$$\text{N accumulation} = N_{input} - N_{output}$$

The N accumulation was remarkably high during the algal bloom in April and May, 1994, when the nitrogen uptake increased dramatically while the denitrifying activity remained at a low level. Thus the surplus nitrogen uptake was evidently caused by assimilative uptake by microphytes and other microorganisms and was apparently accumulated as particulate organic nitrogen in the sediment. During the algal bloom in April–May 1994, the N accumulation was related to the net O_2 release by a factor of 7, with the assumption of a C/O ratio in photosynthesis of 1, a C/N ratio in agreement with the Redfield ratio was found.

During periods with low or no net primary production we still measured a N accumulation in the sediment, and our calculated values of N accumulation might thus not be reliable for net accumulation in microphytobenthos or bacteria. It is possible, however, that an input of organic matter from the terrestrial environment to the stream accounted for a continuous build-up of organic matter in the stream sediment, even when the oxygen budget showed no net primary production. As such terrestrial input might have a high C/N ratio (Enríquez et al. 1993) the bacteria degrading this material would need to assimilate inorganic nitrogen from the overlying water. It is also possible that our N output is underestimated because we did not include efflux of dissolved organic N which may account for a very significant part of the nitrogen flux from sediment to water (Lomstein et al. 1989, Hansen & Blackburn 1991).

In contrast to denitrification which causes a complete removal of chemically bound N, the N accumulated in algal biomass represents only a temporal N retention, delaying the transport to downstream aquatic systems. In their study of sedimentation and resuspension of nitrogen compounds in the Gelbæk, Svendsen & Kronvang (1993) found that the majority of the nitrogen accumulated in the stream during summer was resuspended and exported in the autumn.

Importance of N retention in the stream

As was observed for the Gelbæk, lowland streams often receive large amounts of DIN from the surrounding areas, and serve as the first step in the transport of nitrogen from terrestrial areas to lakes and coastal marine areas. Thus the N retention in streams is of interest for the eutrophication problems in these downstream aquatic ecosystems.

The in-stream retention of N in the Gelbæk due to denitrification and accumulation accounted for approximately 6.65 mmol N m^{-2} d^{-1} which equals 339 kg N ha^{-2} yr^{-1} (the area of the stream bottom accounts for approx. 1 ha). The annual N load in the Gelbæk was 36 t, thus, on an annual basis, less than 1% of the transported N was retained. However, large seasonal variations occurred in the nitrate removal efficiency, primarily caused by the dramatic variations in the N load to the stream system. During spring and summer between 30 and 100% of the N load was retained, but both the load of N and the water discharge were very high during the winter months, and the low N retention by accumulation and denitrifying activity had no significance during this period. The significant reduction in DIN export due to accumulation during the spring and summer months could, however, have a much larger effect than suggested by the low annual

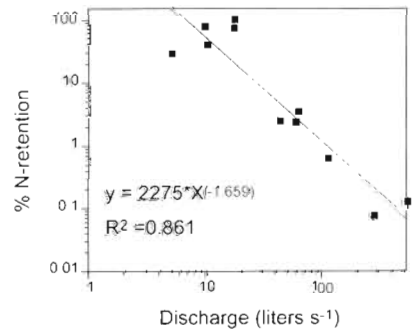

Fig. 6. Double logarithmic plot of percentage N retention vs discharge. N retention is estimated as the sum of denitrification and in-stream N accumulation

percentage N retention. Nitrogen usually limits primary production in near coastal waters (Vitousek & Howarth 1991), and the relatively high N retention in the stream during spring and summer months may reduce the N load and thereby eutrophication in downstream aquatic areas (e.g. estuaries) during this period.

The increase in N load during winter was connected to an increased discharge, and overall the N retention was correlated to the discharge (Fig. 6). Thus a high to moderate N retention efficiency occurred at flows below 20 l s^{-1} while the N retention was reduced to 1–2% when the discharge exceeded 40 l s^{-1}, and at flood occasions with discharges above 200 to 300 l s^{-1} the N retention approached zero. This dramatic decline in the N removal efficiency at increasing discharges was probably caused by erosion of the stream bottom as already discussed, but increased discharges also caused an increase in the water volume in the stream, while the strong currents lowered the residence time. A smaller part of the water thus came into contact with the sediment where both denitrification and N accumulation occurs.

In summary, the N retention in the Gelbæk by both D_w and microphytobenthic consumption is of minor importance on an annual basis, but during the productive period when blooms of phytoplankton may cause heavy eutrophication in lakes and coastal areas (April–September) D_w plus algal assimilation accounted for approximately 25% of the N transported, and may thus have a significant impact on the primary production and thereby the eutrophication in downstream aquatic ecosystems.

Acknowledgements. Lars Svendsen is thanked for delivery of N load and water discharge data. Three anonymous reviewers are acknowledged for critical comments and advice for improving the clarity of the manuscript. This study was supported by grants from the National Science Foundation and Center for Freshwater Research under the Danish Strategic Environmental Research Program.

LITERATURE CITED

Bower CE, Holm-Hansen T (1980) A salicylate-hyperchlorite method for determining ammonia in seawater. Can J Fish Aquat Sci 37:794–798

Christensen PB, Nielsen LP, Revsbech NP, Sørensen J (1989) Microzonation of denitrification activity in stream sediments as studied with a combined oxygen and nitrous oxide microsensor. Appl Environ Microbiol 55:1234–1241

Christensen PB, Nielsen LP, Sørensen J, Revsbech NP (1990) Denitrification in nitrate rich streams, diurnal and seasonal variation related to benthic oxygen metabolism. Limnol Oceanogr 35(3):640–651

Christensen PB, Sørensen J (1988) Denitrification in sediments of lowland streams: regional and seasonal variation in Gelbæk and Rabis bæk, Denmark. FEMS Microbiol Ecol 53:335–344

Cooke JG, White RE (1987) Spatial distribution of denitrification activity in a stream draining an agricultural catchment. Freshwater Biol 18 (3):509–519

Cooper AB, Cooke JG (1984) Nitrate loss and transformations in 2 vegetated headwater streams. NZ J Mar Freshwater Res 18:441–450

Enríquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457–471

Grashoff K, Erhard M, Kremling K (1983) Methods of seawater analysis, 2nd edn. Verlag Chemie, Weinheim

Hansen LS, Blackburn TH (1991) Aerobic and anaerobic mineralization of organic material in marine sediment microcosms. Mar Ecol Prog Ser 75:283–291

Hill AR (1983) Denitrification: its importance in a river draining an intensively cropped watershed. Agric Ecosyst Environ 10:47–62

Hill AR (1988) Factors influencing nitrate depletion in a rural stream. Hydrobiologia 160:111–122

Lomstein BAa, Blackburn TH, Henriksen K (1989) Aspects of nitrogen and carbon cycling in the northern Bering Shelf sediment. I. The significance of urea turnover in the mineralization of NH_4^+. Mar Ecol Prog Ser 57:237–247

Nielsen K, Nielsen LP, Rasmussen P (1995) Estuarine nitrogen retention independently estimated by denitrification rate and mass balance methods: a study of Norsminde fjord, Denmark. Mar Ecol Prog Ser 119:275–283

Nielsen LP (1992) Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol Ecol 86:357–362

Nielsen LP, Christensen PB, Revsbech NP, Sørensen J (1990) Denitrification and photosynthesis in stream sediment studied with microsensor and whole core techniques. Limnol Oceanogr 35:1135–1144

Revsbech NP, Jørgensen BB, Blackburn TH (1980) Oxygen in the sea bottom measured with a microelectrode. Science 207:1355–1356

Risgaard-Petersen N, Rysgaard S, Nielsen LP, Revsbech NP (1994) Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. Limnol Oceanogr 39:573–579

Risgaard-Petersen N, Rysgaard S, Revsbech NP (1993) A sensitive assay for determination of $^{14}N/^{15}N$ isotope distribution in NO_3^-. J Microbiol Methods 17:155–164

Rysgaard S, Christensen PB, Nielsen LP (1995) Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. Mar Ecol Prog Ser 129:111–121

Rysgaard S, Risgaard-Petersen N, Nielsen LP, Revsbech NP (1993) Nitrification and denitrification in lake and estuarine sediments measured by the ^{15}N dilution technique and isotope pairing. Appl Environ Microbiol 59:2093–2098

Ryther JH, Dunstan WH (1971) Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171:1008–1013

Svendsen LM, Kronvang B (1993) Retention of nitrogen and phosphorus in a Danish lowland river system: implications for the export from the watershed. Hydrobiologia 251: 123–135

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115

Wintermanns JFGM, De Mots A (1965) Spectrofotometric characteristics of chlorophyll a and b and their phaerophytins in ethanol. Biochim Biophys Acta 109:448–453

Responsible Subject Editor: T. H. Blackburn, Aarhus, Denmark

Manuscript first received: October 29, 1996
Revised version accepted: February 26, 1997