

Denitrification in sediments of the River Colne estuary, England

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ABSTRACT: The River Colne is a muddy macrotidal estuary on the east coast of England, with strong decreasing gradients of NO_3^- and NH_4^+ down the estuary due to inputs from the river and upstream sewage treatment works. The rates of uncoupled (D_w) and coupled (D_n) denitrification in the sediments at 4 sites were measured monthly by ^{15}N isotope-pairing technique from October 1996 to March 1998. There was a significant spatial and temporal variation in the rates of D_w and D_n . The highest rates were observed at Site 1 and the lowest at Site 4, corresponding to the highest NO_3^- concentrations in the water column and highest organic contents in the sediments at Site 1 and the lowest at Site 4. Short-term kinetic experiments showed that rates of D_w increased with increasing NO_3^- concentration in the water column, up to $500 \mu\text{M NO}_3^-$ at Site 2 (the middle site along the estuary), and were not saturated up to $600 \mu\text{M NO}_3^-$ at Site 1 or $180 \mu\text{M NO}_3^-$ at Site 4. Field observations and kinetics experiments indicated a large sediment potential for D_w at all sites. Nitrate availability limited the rates of D_w and was the main factor regulating the rates of D_w in this estuary. It was calculated that 32 to 44% of total oxidised nitrogen inputs or 20 to 25% of the total inorganic nitrogen inputs (total oxidised nitrogen + ammonium) into the estuary by the River Colne and the sewage treatment works were removed from the estuary by uncoupled denitrification (D_w) and total denitrification ($D_w + D_n$) respectively, in the sediments before they were transported into the North Sea. Microphytobenthos, mainly diatoms, assimilated 4.1% of the ammonium inputs or 2.3% of total inorganic nitrogen inputs into the estuary and temporarily retained the assimilated nitrogen in the estuary. They also affected the rates of denitrification in a complex manner, by regulating the depth of oxygen penetration by photosynthetic oxygen production and respiratory oxygen uptake. Photosynthesis inhibited the rates of D_w but stimulated D_n . The overall effect of microphytobenthos on denitrification was to increase the percentages of D_n in the total denitrification. The loads of nitrogen through the River Colne estuary to the North Sea was significantly attenuated by the removal of nitrate from the water column by D_w and of nitrate produced from ammonium via nitrification in the sediment by D_n .

KEY WORDS: Denitrification · Primary production · Microphytobenthos · Benthic diatoms · Estuaries · Nitrogen

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INTRODUCTION

Denitrification in estuarine sediment is known to be capable of removing significant quantities of nitrate from the water column, providing a sink for nitrogen in aquatic environments, and thereby playing an impor-

tant role in ameliorating the degree of eutrophication in waters subjected to input of nitrogen compounds (Nedwell 1975, Billen et al. 1985, Seitzinger 1988, Rysgaard et al. 1995, Nedwell & Trimmer 1996, Nixon et al. 1996, Ogilvie et al. 1997, Trimmer et al. 1998, Nedwell et al. 1999). Denitrification in sediments is supported both by nitrate diffusing from the overlying water and by nitrate produced within the sediment by nitrification (Vanderborght & Billen 1975, Nishio et al.

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1983, Jenkins & Kemp 1984, Henriksen & Kemp 1988). Diffusion of nitrate from overlying water into an anoxic layer of sediment where denitrification occurs is primarily governed by a concentration gradient determined by the water nitrate concentration and the length of the diffusion path through the oxic zone (Christensen et al. 1990). Nitrification activity in sediment is mainly controlled by the availability of ammonium and oxygen (Hansen et al. 1981, Henriksen et al. 1981).

The quantitative significance of denitrification in attenuating the flux of nitrogen through estuaries into the sea is, as yet, poorly defined. In some estuaries the attenuation of the nitrogen load seems to be significant (Billen et al. 1985, Seitzinger 1988, 1990, Nedwell & Trimmer 1996, Ogilvie et al. 1997), while in others it appears to be small (Nielsen et al. 1995). The degree of attenuation of the nitrogen flux within an estuary is positively related to the freshwater flushing time of the estuary (Balls 1994, Nixon et al. 1996), and can also be influenced by other factors which affect benthic denitrification and nitrification. These factors include bioturbation by benthic infauna (Pelegri et al. 1994, Pelegri & Blackburn 1995, 1996, Gilbert et al. 1998, Svensson 1998), nitrate concentration in the overlying water column and oxygen penetration depth (Christensen et al. 1990), benthic microalgae (Risgaard-Petersen et al. 1994, Rysgaard et al. 1995) and availability of ammonium and oxygen (Blackburn 1996).

In order to better understand the quantitative significance of denitrification in removal of nitrogen from an estuary, environmental factors which may be involved in regulating denitrification need to be investigated. The River Colne is a hypernutrified estuary, with strong decreasing gradients of NO_3^- and NH_4^+ in the water column and organic contents in the sediments down the estuary. The estuary is also characterised by the presence of microphytobenthos on the surface of the sediment. So the purpose of the present study was to investigate the effects of nitrate availability in the water column and the benthic primary producers on denitrification in a North Sea estuary on the east coast of England.

MATERIALS AND METHODS

Sampling locations. The Colne estuary is a small muddy macrotidal (3 to 5 m) estuary (Fig. 1) on the east coast of England, entering the North Sea at Brightlingsea ($51^\circ 45' \text{N}$, $01^\circ 03' \text{E}$). The estuary catchment is 500 km^2 , of which the River Colne drains 300 km^2 , much of which is rich arable land. The estuary exhibits strong increasing gradients of both NO_3^- and NH_4^+ concentrations with distance upstream (King & Nedwell 1987, Ogilvie et al. 1997) as a result of inputs from

the River Colne and a major sewage treatment work at Colchester. Four benthic sampling sites were selected (Fig. 1), covering the full ranges of the estuarine nutrient gradients and sediment types along the estuary. At Sites 2 and 3 in the central stretch of the estuary, where the largest areas of intertidal mud flat were found, both high-shore (mean high water) and low-shore (mean low water) stations (2H, 2L, 3H and 3L) were sampled.

Sediment characteristics. Samples of sediment were taken from the sites for measurements of porosity, sediment particle size and organic content of the sediments. The porosity was determined from the dry weights and wet weights of known volumes of sediment and expressed in terms of $\text{ml H}_2\text{O}$ per ml sediment. The sediment particle size was measured by sieving (mesh size $65 \mu\text{m}$). The sediment organic content was analysed with a CHN analyser (Model 2400, Perkin-Elmer, Beaconsfield, UK) (See Nedwell & Trimmer 1996 for details of methods used).

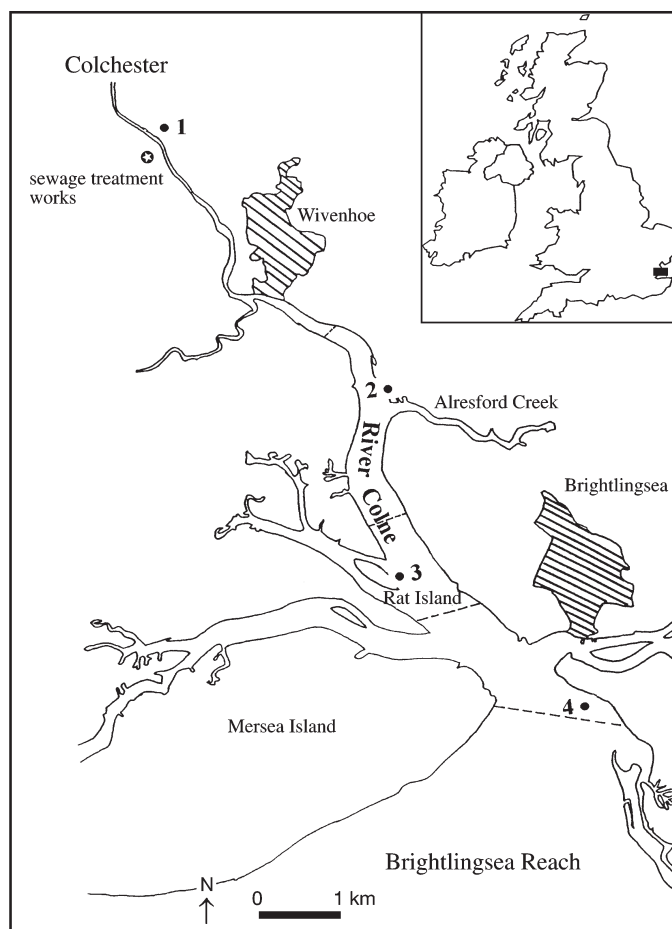


Fig. 1. Map of Colne estuary on east coast of England, showing positions of 4 sampling sites. At Sites 2 and 3 two stations (high shore and low shore) were sampled. Dashed lines divide sectors in estuary

Nutrient concentration in water column. Site water was collected at high tide. Water samples were filtered through glass-fibre filters (GF/F, Whatman, UK) and put into a deep freezer for later analysis. The nitrate, nitrite and ammonium concentrations (Kirkwood 1996) in the water samples were analysed by a nutrient auto-analyser (Skalar Analytical B.V., Breda, The Netherlands). Salinity was measured by a refractory meter (Leica, New York, USA).

Chlorophyll *a* content in surface sediment. The sediment samples from the top 50 mm layer of sediments were collected for chlorophyll *a* analysis (see Underwood et al. 1995). About 100 mg of freeze-dried sediment was extracted in 4 ml MgCO_3 -buffered 100% methanol for 24 h at 4°C in the darkness. Following centrifugation for 15 min at $2000 \times g$, absorbances of the supernatant was measured at 665 and 750 nm using a spectrophotometer (CECIL, Cambridge, UK) followed by acidification. Chlorophyll *a* concentration was calculated by the equation of Stal et al. (1984).

Oxygen flux across sediment-water interface. Triplicate sediment cores (~30 cm deep) were collected from each site with Perspex core tubes (65 cm long \times 8 cm i.d.). On return to the laboratory the water volume in the core tube above the sediment was adjusted to 500 ml, and the cores were immersed in aerated site water at *in situ* water temperature to re-equilibrate overnight. Next day, the rates of oxygen uptake or evolution were measured in the light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$; this is the light used for all experiments in this study) and dark at *in situ* temperature. 'Light' incubations were performed at a time corresponding to the time of low tide, so the natural migratory cycle of benthic diatoms would ensure that they were present at the surface of the sediment when the measurements were made. The water column in each tube was stirred with an induction motor driving a magnetic follower (Rank Brothers Ltd, Cambridge, UK) at 300 rpm. (Preliminary experiments showed that benthic oxygen uptake was unaffected by stirring rates over the range 300 to 900 rpm). Triplicate water samples were collected at the beginning and end of the incubation from each core tube, and the oxygen concentrations were measured by Winkler titration (Strickland & Parsons 1972).

Vertical concentration profiles of dissolved oxygen in sediments. These were determined in sediment cores in the light and dark with a very fine, glass oxygen microelectrode (home-made, not commercially available). The microelectrode was calibrated in aerated site water (100% of O_2 saturation) and in water with added sodium dithionite (0% of O_2 saturation). It was then introduced into water overlying the sediment and down into sediment. The vertical movement of the electrode was controlled by a stepping motor linked to a micromanipulator. The stepping motor was con-

trolled by a computer programme (TiePie Engineering, Leeuwarden, The Netherlands) to give 20 μm depth steps at 2 s intervals.

Measurement of denitrification with $^{15}\text{NO}_3^-$. The isotope-pairing technique (Nielsen 1992) was used to measure sedimentary denitrification from both external NO_3^- , derived by transport into the sediment from the water column (D_w), and from NO_3^- generated within the sediment by nitrification (D_n). Cores of sediment (~10 cm deep) were collected in Perspex tubes (3.4 cm i.d. \times 22 cm length) from each site. On return to the laboratory, the water above the sediment was carefully replaced with site water. The cores were put in an incubation tank at *in situ* water temperature and submerged in site water which was vigorously bubbled with air overnight to re-equilibrate. Next day, the rate of denitrification was measured.

Time-course experiments: The measurement of denitrification by the isotope-pairing technique requires addition of $^{15}\text{NO}_3^-$ into the water column overlying sediment. It takes some time for the added $^{15}\text{NO}_3^-$ to mix homogeneously with the $^{14}\text{NO}_3^-$ in the water column and in the sediment. To determine the optimum incubation time for the measurement, time-course experiments were carried out. The incubation time began on addition of 50 μM (Site 4) and 100 μM (Site 1) of $\text{Na}^{15}\text{NO}_3^-$ (99.3% ^{15}N ; Europa Scientific, Crewe, UK) into the water column (where the unlabelled nitrate concentrations were 21 and 295 μM , respectively, for Sites 4 and 1) of each core, and the core was immediately stoppered. The water columns were stirred at 60 rpm during incubation with small magnetic followers (2 cm length) in the middle of the water column to maintain mixing. Cores were incubated for 15, 30, 45, 60, 120, or 180 min in a water bath at *in situ* temperature in the dark. The dissolved O_2 in the water column never decreased below 80% of air saturation. At the end of each incubation, the stopper was removed from each core tube and ZnCl_2 solution (0.5 ml of 50% w/v) added to the water column to stop microbial activity; then the sediment and water column were stirred to mix. A sample of the resultant slurry was immediately removed with a syringe and transferred to a 12.5 ml exetainer (Labco, High Wycombe, UK). A further 100 μl of ZnCl_2 solution was added to the exetainer, which was then sealed. Four ml of water sample in the exetainer was replaced by ultra pure helium. Nitrogen gas in the slurry sample diffused into the helium headspace of the exetainer. The gas samples from the helium headspace were analysed for $^{28}\text{N}_2$, $^{29}\text{N}_2$ and $^{30}\text{N}_2$ using a Europa dual-inlet mass spectrometer fitted with a Europa Automatic Nitrogen and Carbon Analyser system (Europa Automatic Nitrogen, Crewe, UK), located in the NERI laboratory in Silkeborg, Denmark.

The time-course experiments showed that after approx. 45 min at Site 1 and 30 min at Site 4, the measured dinitrogen gas production became linear with incubation time. For later measurements, 30 min was given after addition of $^{15}\text{NO}_3^-$ into the water column for the labelled and unlabelled nitrate to mix homogeneously before starting incubation.

It is assumed that addition of $^{15}\text{NO}_3^-$ must not alter the *in situ* rates of coupled- and uncoupled denitrification based on $^{14}\text{NO}_3^-$. Thus, it is necessary to verify this assumption and to optimise $^{15}\text{NO}_3^-$ concentrations added. Two approaches were adopted for this purpose. One was to vary $^{15}\text{NO}_3^-$ concentrations but keep $^{14}\text{NO}_3^-$ concentration constant; the other was to vary $^{14}\text{NO}_3^-$ concentrations but keep $^{15}\text{NO}_3^-$ concentration constant. The point is that, ideally, the rate of denitrification based on *in situ* $^{14}\text{NO}_3^-$ concentration should be independent of the $^{15}\text{NO}_3^-$ concentration added.

Varying $^{15}\text{NO}_3^-$ concentrations but keeping $^{14}\text{NO}_3^-$ concentration constant: A series of $^{15}\text{NO}_3^-$ concentrations (10, 20, 50, 75, 100, 250 μM) were added into the water column (where the *in situ* $^{14}\text{NO}_3^-$ concentrations were 21 and 295 μM , respectively, for Sites 4 and 1) of each core, which was left unstoppered for approx. 30 min for labelled $^{15}\text{NO}_3^-$ and unlabelled $^{14}\text{NO}_3^-$ to mix homogeneously and equilibrate into the denitrifying layer of the sediments. Each core was then stoppered and incubated for 2 h at *in situ* temperature in the dark.

Varying $^{14}\text{NO}_3^-$ concentrations but keeping $^{15}\text{NO}_3^-$ concentration constant: A solution of $^{14}\text{NO}_3^-$ was added into the overlying water column (nitrate-free artificial seawater) to make a series of the required concentrations, and labelled $^{15}\text{NO}_3^-$ was also added into the cores collected from Site 4 to give constant final concentrations of $^{15}\text{NO}_3^-$ of 50 μM . The cores were left unstoppered for 30 min to equilibrate, then stoppered and incubated for 2 to 3 h at *in situ* temperature in the dark.

Monthly *in situ* rates of denitrification: Seven cores were collected from each site every month from October 1996 to March 1998. Of these, 2 cores were used for reference controls and the remaining 5 for incubation using *in situ* water and at *in situ* temperature in the dark. From April 1997 to March 1998, a further set of 7 cores were collected from each site and used for incubation in the light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$). Immediately prior to the start of incubation, 5 ml of column water was withdrawn from each reference core and transferred into 7 ml bijoux vials (Bibby Sterilin, Stone, UK) and immediately frozen for later nitrate analysis. To the remaining 5 cores $^{15}\text{NO}_3^-$ was added and they were left for 30 min to reach equilibrium. Immediately prior to closing the core tubes with stoppers, 2 ml of

column water was removed from each core for later nitrate analysis. The ratio of unlabelled and labelled nitrate was calculated by the difference before and after addition of labelled nitrate. Incubation usually lasted 2 to 4 h, depending on the incubation temperature.

Short-term kinetic experiments: Kinetics of denitrification were examined by measurements of denitrification of sediments samples collected from Sites 1 and 2H at a series of nitrate concentrations (4 replicates for each concentration). After incubation in the dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$), a 12 ml water sample was taken from each core for measurement of O_2 concentration, then ZnCl_2 solution (0.5 ml of 50% w/v) was added to the water column and the sediment and water column were stirred to mix. A sample of slurry was removed for isotope measurement. Chlorophyll *a* content in the top 50 mm layer of the sediments was also measured (sediment samples for chlorophyll *a* were collected using separate sediment cores). More details about kinetic experiments are given in the legend to Fig. 9.

The rates of denitrification were calculated according to the equations of Nielsen (1992): $D_{15} = p(^{15}\text{N}^{14}\text{N}) + 2p(^{15}\text{N}^{15}\text{N})$, and $D_{14} = p(^{15}\text{N}^{14}\text{N}) + 2p(^{14}\text{N}^{14}\text{N})$, where D_{15} and D_{14} = rates of denitrification based on $^{15}\text{NO}_3^-$ and $^{14}\text{NO}_3^-$, respectively; and $p(^{14}\text{N}^{14}\text{N})$, $p(^{15}\text{N}^{14}\text{N})$ and $p(^{15}\text{N}^{15}\text{N})$ = rates of production of labelled and unlabelled N_2 species. Because the $p(^{14}\text{N}^{14}\text{N})$ cannot be readily measured, estimation of D_{14} was obtained from: $D_{14} = D_{15} \times p(^{15}\text{N}^{14}\text{N}) / 2p(^{15}\text{N}^{15}\text{N})$.

The proportion of D_{14} supported by unlabelled NO_3^- from the water column (D_w) was calculated from: $D_w = D_{15} \times f / (1 - f)$, where f = mole fraction of $^{14}\text{NO}_3^-$ in the water column. The coupled nitrification-denitrification (D_n) was calculated by difference as: $D_n = D_{14} - D_w$.

RESULTS

Characteristics of sampling sites in Colne estuary

Sediment characteristics for the 4 sites are shown in Table 1. Sediments at Sites 1, 2 and 3 were fine silt, while at Site 4, sediments were clay with a very thin layer of fine muddy sand on the surface. The highest organic carbon content and porosity of the sediments were at Site 1, the lowest at Site 4. The highest water temperature was 24°C in August and the lowest 1.5°C in early February. Salinity of the water at Site 1 was variable, in the range of 2 to 17‰, depending upon river flow. Salinity at Site 2 was less variable, in the range of 20 to 32‰. Salinity at Sites 3 and 4 was rather stable, in the range of 28 to 34‰, with a mean value of 32‰.

Table 1. Organic carbon content, porosity, and particle size of top 0.5 cm of sediments at 4 sites in Colne estuary (data are ranges of 4 replicates)

| Parameter | Date | Site | | | |
|---|------------------|------------------------|------------------------|------------------------|------------------------|
| | | 1 | 2 | 3 | 4 |
| Organic carbon (% dry wt) | Jul 97 Feb 98 | 2–3 3–3.7 | 1.8–2 2–3 | 1.5–2 1.5–3 | 0.4–0.5 1–2 |
| Porosity (ml H ₂ O ml ⁻¹ sediment) | Jul 97 Feb 98 | 0.75–0.92 0.72–0.75 | 0.76–0.82 0.69–0.85 | 0.65–0.80 0.66–0.82 | 0.55–0.68 0.53–0.70 |
| Particles size (silt/clay fraction <65 µM) | Feb 98 | 87–98% | 80–95% | 89–91% | 35–43% |

Denitrification

Varying ¹⁵NO₃⁻ concentrations but keeping ¹⁴NO₃⁻ concentration constant

Rates of D_{14} (denitrification based on ¹⁴NO₃⁻) were not significantly affected by the addition of 75 to 250 µM ¹⁵NO₃⁻ for Site 1 or 25 to 250 µM for Site 4. Thus, for the monthly measurements, usually 50 µM ¹⁵NO₃⁻ was used for labelling for cores from Sites 2, 3 and 4, but 100 µM ¹⁵NO₃⁻ was used for cores from Site 1. In certain months, when nitrate concentrations in site water were high, the added ¹⁵NO₃⁻ concentration was increased accordingly to make the enrichment of ¹⁵NO₃⁻ not <30% of the unlabelled nitrate.

Varying ¹⁴NO₃⁻ concentrations but keeping ¹⁵NO₃⁻ concentration constant

Rates of D_{14} increased linearly with the increase of ¹⁴NO₃⁻ concentration from 10 to 180 µM in the water column, while rates of D_{15} remained constant (Fig. 2A). This means that D_{14} was independent of ¹⁵NO₃⁻ and D_{15} was independent of ¹⁴NO₃⁻. In other words, addition of labelled ¹⁵NO₃⁻ into the water column did not kinetically affect D_{14} . Uncoupled denitrification (D_w) increased linearly with the ¹⁴NO₃⁻ concentration in the water column, while coupled nitrification-denitrification (D_n) was constant, and unaffected by the addition of ¹⁵NO₃⁻ and ¹⁴NO₃⁻ to the water column (Fig. 2B). These results showed that (1) the rates of coupled nitrification-denitrification were independent of nitrate concentration in the water column, and (2) the measured rates of uncoupled denitrification were independent of ¹⁵NO₃⁻ added.

Results of monthly survey

These results showed that the rates of uncoupled denitrification (D_w) decreased down the estuary to the mouth, from Sites 1 to 4, following the decreasing

nitrate concentration gradients in the water column from Sites 1 to 4. Nitrate concentrations in the water column were in the range 76 to 1171 µM at Site 1, 16 to 504 µM at Site 2, and 3.3 to 94 µM at Sites 3 and 4.

At head (Site 1) of estuary. Rates of uncoupled (D_w) and coupled (D_n) denitrification in both dark and light were the highest among all sites in this estuary (average dark rates of D_w 530 µmol N m⁻² h⁻¹ and D_n 179 µmol N m⁻² h⁻¹, and average light rates of D_w 1140 µmol N m⁻² h⁻¹ and D_n 273 µmol N m⁻² h⁻¹ from

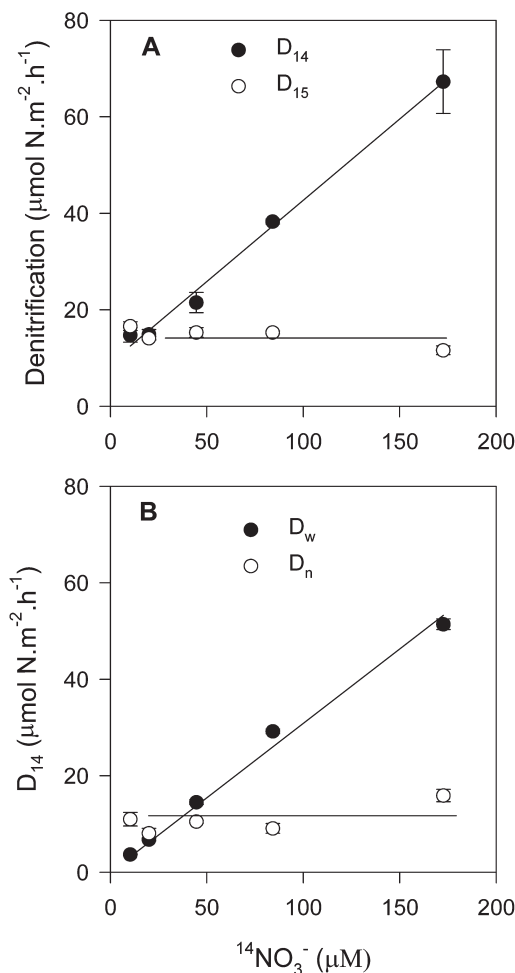


Fig. 2. Denitrification rates vs ¹⁴NO₃⁻ concentration with constant 50 µM ¹⁵NO₃⁻ concentration in overlying water column for sediment at Site 4. Incubations were at 15°C in darkness using artificial seawater (30‰ S) on 9 December 1997. Bars indicate SE (n = 4). (A) Rates of D_{14} ($D_w + D_n$) and D_{15} (denitrification based on ¹⁵NO₃⁻ in water column); (B) rates of D_w (denitrification based on ¹⁴NO₃⁻ in water column) and D_n (coupled nitrification-denitrification)

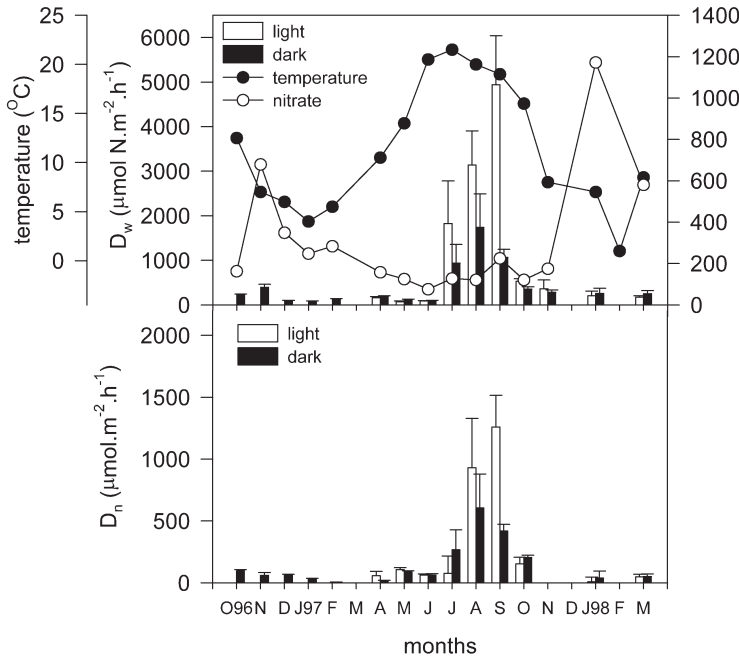


Fig. 3. Rates of uncoupled denitrification (D_w) and coupled nitrification-denitrification (D_n) in sediments measured in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using *in situ* water and *in situ* temperature, nitrate concentration in overlying water column, and water temperature at Site 1 of River Colne estuary from October 1996 to March 1998. Bars indicate SE ($n = 5$)

April 1997 to March 1998: Fig. 3). There was a large increase in the rates of denitrification in the summer of 1997 at the same time as a significant increase in both infauna biomass and the number of individual polychaetes in the sediment (Cooper 1999). In July, August and September 1997, the rates of D_w in the light were significantly higher than those in the dark (ANOVA, $p < 0.05$). The reason for this is unclear. The rates of D_w measured in the light and dark for the other months were not significantly different ($p > 0.05$). Rates of D_n measured in the light and dark were not significantly different from April 1997 to March 1998 ($p > 0.05$).

Uncoupled denitrification (D_w) represented the larger proportion (40 to 100 %) of the total denitrification, while coupled denitrification (D_n) was 0 to 60 % (Fig. 4), because of the high nitrate concentration in the water column and oxygen limitation on nitrification. There was no significant difference in percentages of D_n in the dark compared with those in the light at Site 1 ($p > 0.05$).

In the middle of the estuary (Sites 2 and 3). Rates of D_w at Site 2 lay between those at Sites 1 and 4, corresponding to intermediate concentrations of nitrate (16 to $76 \mu\text{M}$) in the water column at this site during October 1996 to November 1997. High-shore (2H) and low-shore (2L) sites showed similar rates of D_w in the dark, 15.3 to $98.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$ at the high-shore (Fig. 5)

and 14.3 to $64.7 \mu\text{mol N m}^{-2} \text{h}^{-1}$ at the low-shore (data not shown) sites. In January and March 1998, nitrate concentrations in the water column were exceptionally high ($504 \mu\text{M}$ in January and $227 \mu\text{M}$ in March) due to the high rainfall, resulting in much higher rates of D_w at Sites 2H and 2L. This indicates that nitrate availability normally regulated the rate of D_w . The rates of D_n in the dark at the high-shore site were not significantly different ($p > 0.05$) from those at the low-shore site, so there was no significant spatial difference in the rates of D_w or D_n at Site 2. There was no clear seasonal pattern in the variation of D_w or D_n at Site 2.

At Site 2H, neither D_w nor D_n in the dark were significantly different from those in the light ($p > 0.05$) (Fig. 5). The percentages of D_n in the dark and light showed no significant difference ($p > 0.05$) (Fig. 4). At Site 2L, the rates of D_n in the dark were not significantly different from those in the light ($p > 0.05$), but the rates of D_w in the dark were significantly higher than those in the light ($p < 0.05$). The percentages of D_n in the light were

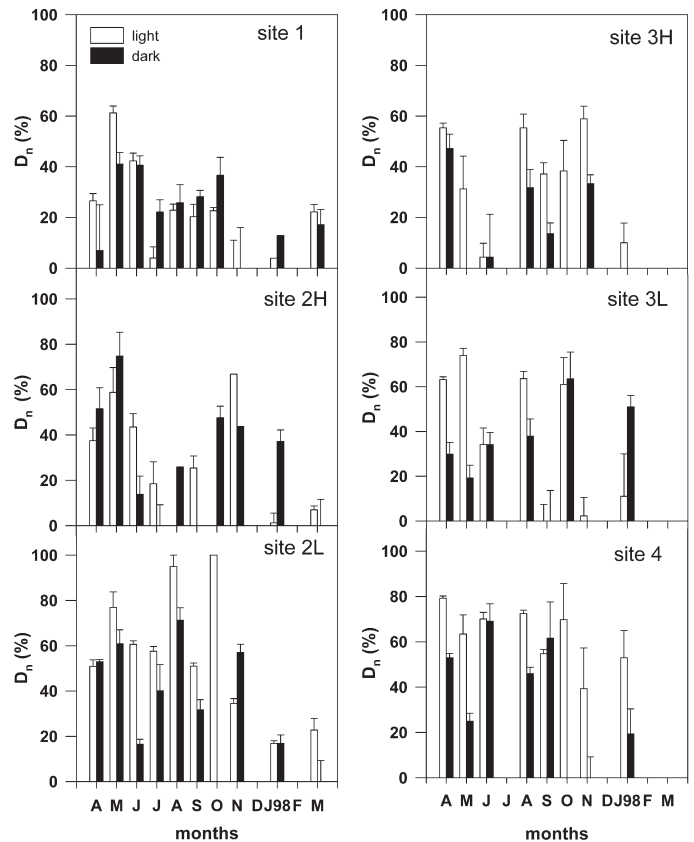


Fig. 4. Percentages of D_n in total denitrification ($D_w + D_n$) at all sites of the River Colne estuary from April 1997 to March 1998. Bars indicate SE ($n = 5$). H: high shore; L: low shore

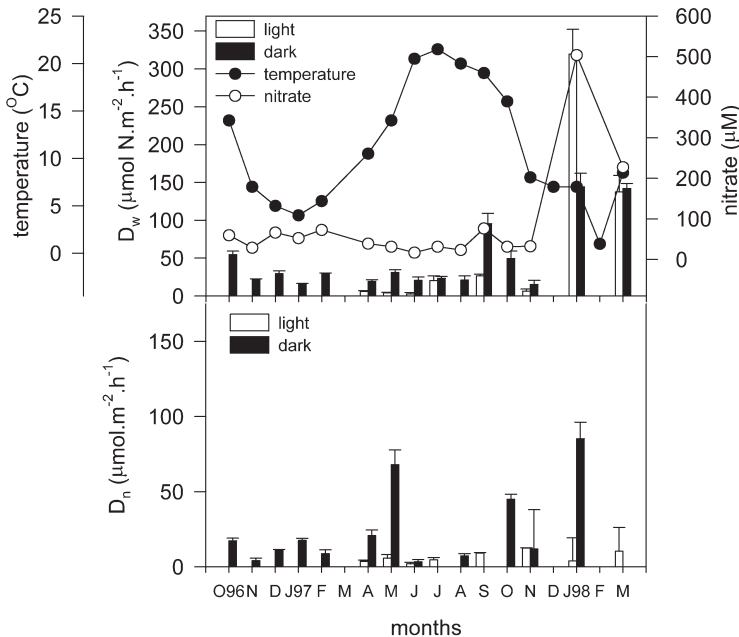


Fig. 5. Rates of uncoupled denitrification (D_w) and coupled nitrification-denitrification (D_n) in sediments measured in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using *in situ* water and *in situ* temperature, nitrate concentration in overlying water column, and water temperature at Site 2H of River Colne estuary from October 1996 to March 1998. Bars indicate SE ($n = 5$)

significantly higher than in the dark ($p < 0.05$) (Fig. 4). This suggests that oxygen produced by the benthic biofilms in the light may inhibit D_w at Site 2L.

At Site 3, the rates of D_w in the dark at both high-shore and low-shore were similar ($p > 0.05$: average 10.2 , range 1.9 to $23.5 \mu\text{mol N m}^{-2} \text{h}^{-1}$, at high-shore [Fig. 6] site and average 9.4 , range 1.1 to $28 \mu\text{mol N m}^{-2} \text{h}^{-1}$ at low shore site [data not shown]). The rates of D_n in the dark at high-shore (average 2.4 , range 0 to $6.6 \mu\text{mol N m}^{-2} \text{h}^{-1}$) site were significantly lower ($p > 0.05$) than at low-shore (average 6 , range 0 to $15.7 \mu\text{mol N}_2 \text{m}^{-2} \text{h}^{-1}$) site. Neither D_w nor D_n in the light at high-shore or low-shore sites was significantly different ($p > 0.05$). The percentages of D_n at both high-shore and low-shore sites were significantly higher in the light than in the dark ($p < 0.05$, Fig. 4). There was no clear seasonal pattern in the variation of D_w or D_n at Site 3.

At the mouth (Site 4) of the estuary. Rates of D_w were the lowest among all sites in the estuary, in the ranges 1.2 to $13.1 \mu\text{mol N m}^{-2} \text{h}^{-1}$ in the dark (October 1996 to November 1997) and 1.4 to $13.9 \mu\text{mol N m}^{-2} \text{h}^{-1}$ in the light (April 1997 to November 1997), corresponding to the lowest

nitrate concentrations of 3 to $38 \mu\text{M}$ in the overlying water (Fig. 7). However, in January 1998, due to high rainfall, nitrate concentrations in the water column rose to $94 \mu\text{M}$. Correspondingly, the rates of denitrification increased up to $69.5 \mu\text{mol N m}^{-2} \text{h}^{-1}$ in the dark and $23.3 \mu\text{mol N m}^{-2} \text{h}^{-1}$ in the light. This suggested that nitrate availability in the water column was limiting the rate of D_w at Site 4. Further evidence that water-column nitrate limited the rate of benthic denitrification at Site 4 was supplied by the nitrate supplementation experiment. A linear relationship between D_w and nitrate concentration in the overlying water was observed up to nitrate concentrations of $180 \mu\text{M}$ in dark incubation (Fig. 2) at this site.

From April 1997 to January 1998, the rates of D_w in the dark and light were not significantly different ($p > 0.05$), but the rates of D_n in the light were significantly higher than those in the dark ($p < 0.05$), suggesting that oxygen produced by the biofilms may stimulate D_n via enhanced nitrification. In most months, the percentages of D_n were significantly higher in the light than in the dark ($p < 0.05$). The coupled denitrification at this site represented a larger proportion of the total denitrification (25 to 80 %) than at Site 1 (Fig. 4).

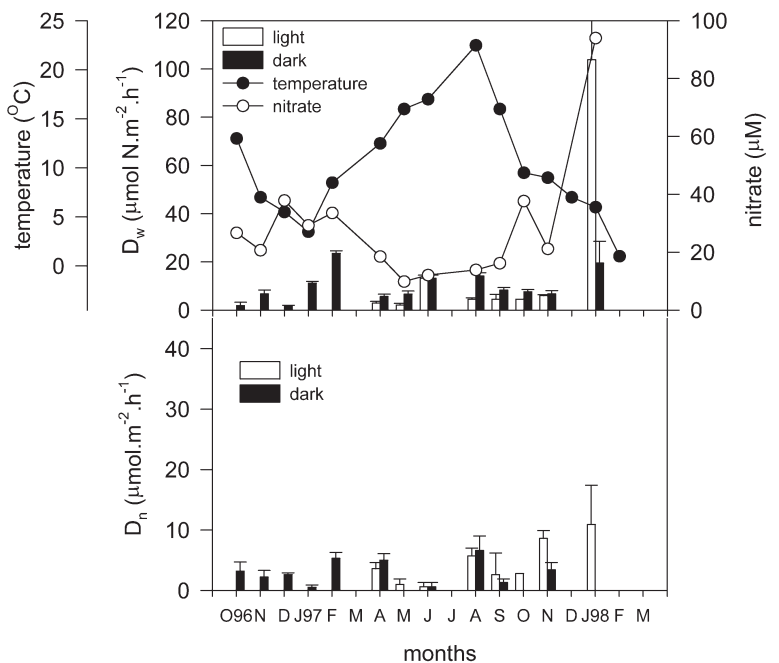


Fig. 6. Rates of uncoupled denitrification (D_w) and coupled nitrification-denitrification (D_n) in sediments measured in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using *in situ* water and *in situ* temperature, nitrate concentration in overlying water column, and water temperature at Site 3H of River Colne estuary from October 1996 to March 1998. Bars indicate SE ($n = 5$)

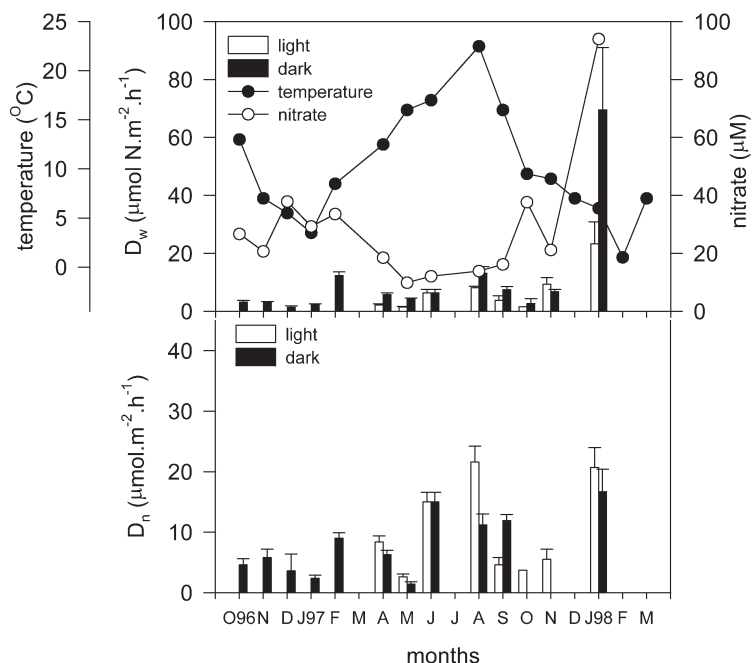


Fig. 7. Rates of uncoupled denitrification (D_w) and coupled nitrification-denitrification (D_n) in sediments measured in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using *in situ* water and *in situ* temperature, nitrate concentration in overlying water column, and water temperature at Site 4 of River Colne estuary from October 1996 to March 1998. Bars indicate SE ($n = 5$)

Oxygen fluxes across sediment/water interface

At Site 1, there was a net uptake of oxygen by the sediment both in the dark and light during most months, but a net output of oxygen in the light on 4 occasions (Fig. 8). Oxygen uptake rates by the sediment were linearly correlated to the water temperature in the dark ($r^2 = 0.80$).

At Sites 2, 3 and 4, there were generally net oxygen fluxes from the sediment into the overlying water in the light, arising from the photosynthesis of the diatom-dominated benthic biofilms on the surface of the sediment, and net oxygen uptake in the dark (Fig. 8). There were no clear seasonal patterns in the rates of oxygen flux at Sites 2, 3 and 4.

Short-term kinetic experiments on effect of microphytobenthic biofilms on denitrification

At Site 1, the chlorophyll *a* content in the top 50 mm layer of the sediment samples (collected in April 1998) was $29.9 \pm 3.3 \mu\text{g g}^{-1}$ dry sediment (mean \pm SE, $n = 5$). The net O_2 output by the diatom-dominated biofilms was $4 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ in the light, and the net O_2 uptake by the sediment was $7.2 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ in

the dark. The rates of D_w increased linearly with increasing nitrate concentration in the water column (Fig. 9A). The slopes of the linear regression of the rates of D_w versus nitrate concentrations showed no significant difference in the light and dark ($p > 0.05$). The measured rates of D_n (Fig. 9B) in the light (126.6 ± 17.8 , $n = 20$) were significantly higher than in the dark (18.8 ± 6.7 , $n = 20$) ($p < 0.05$), but unaffected by NO_3^- concentration. This means that the D_n was stimulated but the D_w unaffected by O_2 produced by diatoms in the light at this site. The results also show that there was a big potential in denitrification at this site, since even up to a concentration of $600 \mu\text{M}$ nitrate the rates of D_w were still not saturated.

At Site 2H, the chlorophyll *a* content in the top 50 mm layer of sediment samples (collected in July 1998) was $50.2 \pm 3.6 \mu\text{g g}^{-1}$ dry sediment (mean \pm SE, $n = 5$). High rates of net oxygen output ($8.1 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$) were observed in the light arising from the photosynthesis of the benthic biofilms, but net oxygen uptake ($2.45 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$) by the sediment was measured in the dark. The rates of D_w responded to changes in nitrate con-

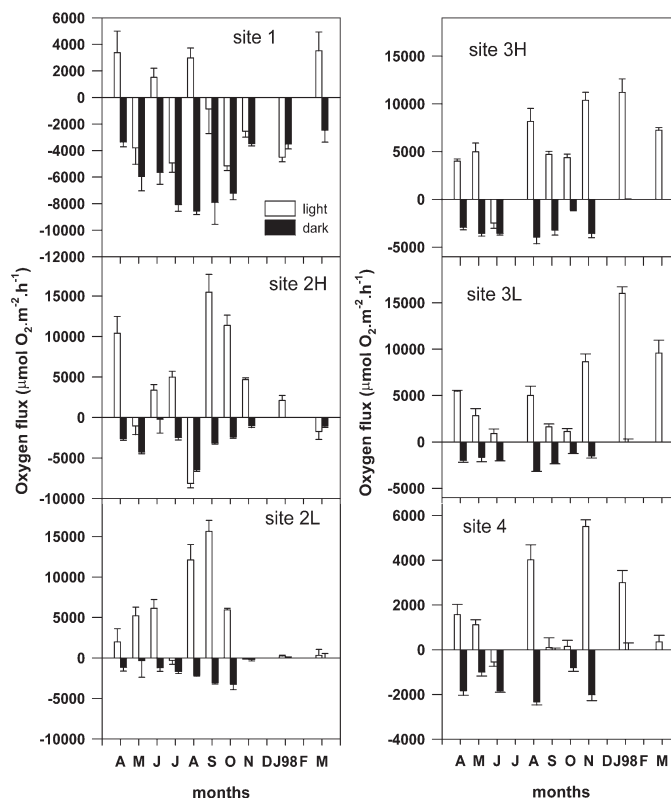


Fig. 8. Rates of oxygen flux across sediment/water interface measured in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using *in situ* water and *in situ* temperature at all sites of River Colne estuary from April 1997 to March 1998. Bars indicate SE ($n = 3$)

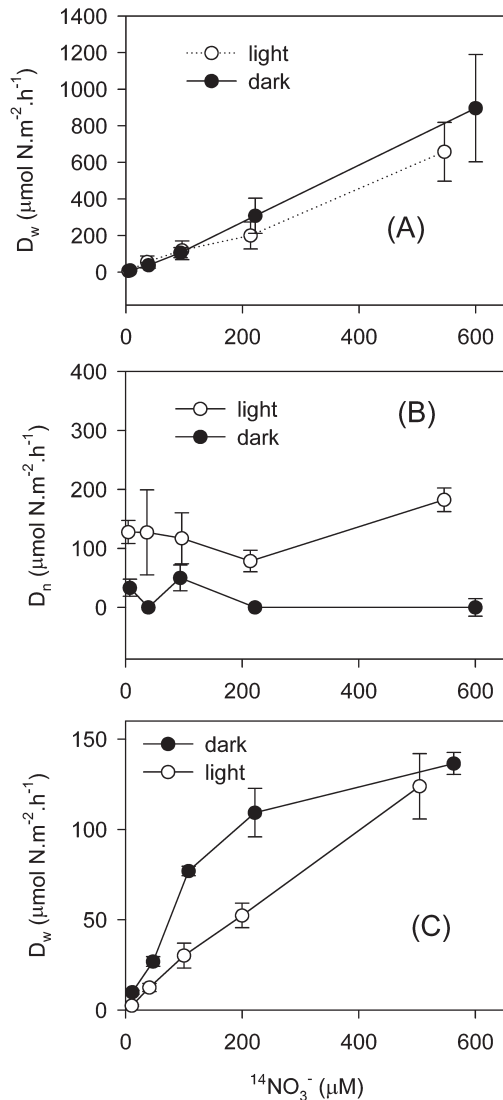


Fig. 9. Rates of D_w and D_n vs $^{14}\text{NO}_3^-$ concentration with constant $110 \mu\text{M}$ $^{15}\text{NO}_3^-$ concentration in water column for sediments at Sites 1 and 2H of River Colne estuary. Incubations were at 15°C in dark and light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) using artificial seawater (15‰ S) on 29 April 1998 for Site 1 and 29 July 1998 for Site 2H. Bars indicate SE ($n = 4$). (A) Rates of D_w for Site 1; (B) rates of D_n for Site 1; and (C) rates of D_w for Site 2H

centrations, with typical Michaelis-Menten-type saturation kinetics in the dark, but first-order kinetics in the light (Fig. 9C). Below $500 \mu\text{M}$ nitrate, the rate of D_w in the dark was significantly higher than in the light ($p < 0.05$). At $500 \mu\text{M}$ nitrate, the rates of D_w in the dark and light became similar. The rates of D_n in the dark were not significantly ($p > 0.05$) different from those in the light, and were not affected by increase of nitrate concentration in the water column (data not shown). The results indicate that at Site 2H the effect of benthic biofilms on denitrification was to inhibit D_w .

At Site 2H, kinetic experiments also show that the rates of denitrification seemed not to be saturated under $500 \mu\text{M}$ (Fig. 10). The Eadie-Hofstee-transformed version of the Michaelis-Menten equation ($[\text{NO}_3^-]/D_{14} = [\text{NO}_3^-]/D_{\text{max}} + K_m/D_{\text{max}}$, Day et al. 1989) was used to deduce the maximum rate of denitrification ($D_{\text{max}} = 222 \mu\text{mol N m}^{-2} \text{h}^{-1}$) and the half-saturation concentration of nitrate ($K_m = 187.8 \mu\text{M}$) in the dark. This further demonstrated that there was a large potential for denitrification at this site, and the rates were usually limited by low availability of nitrate throughout the year because the nitrate concentrations in site water were usually below the K_m (Fig. 10).

DISCUSSION

Isotope-pairing technique for denitrification measurements

The isotope-pairing technique (Nielsen 1992) for denitrification measurements allows quantification of both coupled nitrification-denitrification (D_n) and uncoupled denitrification supported by nitrate diffusing into the sediment from the overlying water column (D_w). However, correct measurement of denitrification by this technique requires fulfilment of 4 assumptions: namely that the added $^{15}\text{NO}_3^-$ must mix homogeneously with the $^{14}\text{NO}_3^-$ in the water column and in the sediment; the addition of $^{15}\text{NO}_3^-$ must not alter the *in situ*

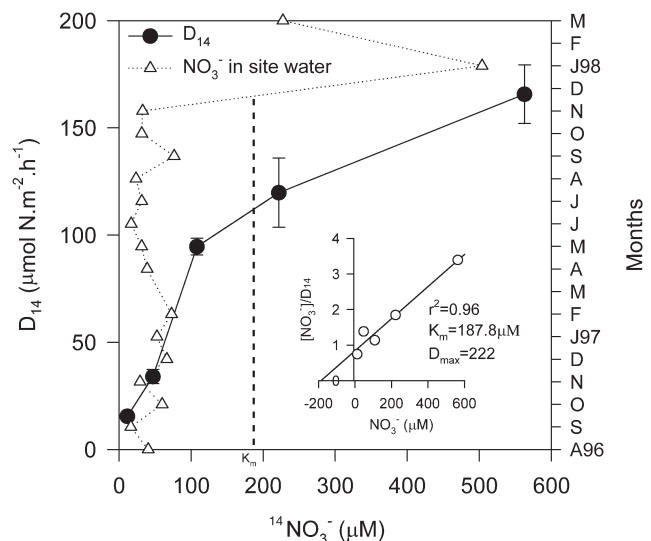


Fig. 10. Rates of denitrification vs $^{14}\text{NO}_3^-$ concentration with constant $110 \mu\text{M}$ $^{15}\text{NO}_3^-$ concentration in overlying water column for sediment at Site 2H. Incubations were at 15°C in darkness using artificial seawater (15‰ S) on 19 February 1997, and nitrate concentrations of *in situ* water from August 1996 to March 1998. Bars indicate SE ($n = 4$)

rates of coupled and uncoupled denitrification; the isotope fractionation can be neglected; and the 2 isotopes ($^{14}\text{NO}_3^-$ and $^{15}\text{NO}_3^-$) diffuse similarly, i.e. according to their abundance ratio.

Kinetic experiments (Fig. 2A) demonstrated that the rates of D_{15} were constant even with increasing $^{14}\text{NO}_3^-$ concentration in the water column and the ratios of $^{14}\text{N}^{15}\text{N}$ to $^{15}\text{N}^{15}\text{N}$ increased linearly with increase of $^{14}\text{NO}_3^-$ concentration, thus verifying the assumption of homogeneous mixture of added $^{15}\text{NO}_3^-$ with $^{14}\text{NO}_3^-$. The added $^{15}\text{NO}_3^-$ equilibrated rapidly with $^{14}\text{NO}_3^-$, in only 30 to 40 min. Several authors (Broast et al. 1988, Nielsen 1992, Pelegri et al. 1994, Risgaard et al. 1994, Rysgaard et al. 1995) have demonstrated that when the production of $^{14}\text{N}^{15}\text{N}$ was less than that predicted on the assumption of homogeneity, the *in situ* denitrification could be underestimated.

There was a first-order kinetic relationship between the rates of D_w and the $^{14}\text{NO}_3^-$ concentrations in the water column when a constant $50 \mu\text{M } ^{15}\text{NO}_3^-$ was added at Site 4 (Fig. 2B) and $110 \mu\text{M } ^{15}\text{NO}_3^-$ added at Site 1 (Fig. 9A). The rates of D_n were independent of $^{14}\text{NO}_3^-$ concentrations in the water column with $50 \mu\text{M } ^{15}\text{NO}_3^-$ added at Site 4 (Fig. 2B) and with $110 \mu\text{M } ^{15}\text{NO}_3^-$ added at Site 1 (Fig. 9B). These results indicate that addition of $^{15}\text{NO}_3^-$ affected neither D_w (based on *in situ* $^{14}\text{NO}_3^-$) nor D_n . The second assumption was thus satisfied.

Discrimination against ^{15}N in the process of denitrification was reported to result in an error $<0.5\%$, and can therefore be disregarded (Middelburg et al. 1996). The validity of the 4th assumption that the 2 isotopes ($^{14}\text{NO}_3^-$ and $^{15}\text{NO}_3^-$) diffuse similarly has generally been accepted by the users of the isotope-pairing technique, although it has been questioned in some circumstances (Middelburg et al. 1996).

Field and laboratory work

The rates of D_w decreased from Sites 1 to 4, following the NO_3^- concentrations in the water column. At Site 1, where the nitrate concentrations were the highest among all sites in this estuary, the rates of D_w were also the highest, but not correlated to the nitrate concentrations in the water column (Fig. 3). For example, in the summer of 1997, the rates of D_w were significantly higher than other months ($p < 0.05$), although the nitrate concentrations in the water column were lower than in the winter. It is likely that the increase in the rates of denitrification at this time was due to the increased activities of the benthic infauna through burrow building, bioturbation and irrigation (Rhoads 1974, Aller 1982, Kristensen 1984, Pelegri et al. 1994, Cooper 1999). However, at Sites 2, 3 and 4, the rates of

D_w responded to the changes in nitrate concentrations in the water column with typical Michaelis-Menten kinetics from October 1996 to March 1998 (Fig. 11). The nitrate concentrations in the water column at Site 2 were usually below the K_m ($187.8 \mu\text{M}$; Fig. 10) and were even lower at Sites 3 and 4 (Figs. 6 & 7). These data indicate that in most of the estuary (Sites 2, 3, and 4) availability of nitrate limited the rates of D_w , but not at Site 1.

It must be emphasized that denitrification is not only determined by nitrate concentration in the water column, but also by organic carbon content in the sediment. The present study showed that at a concentration of nitrate below the saturation concentration, the rates of D_w were different at a given nitrate concentration (for instance, $100 \mu\text{M } \text{NO}_3^-$) at different sites ($100 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at Site 1 (Fig. 9A), $70 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at Site 2 (Fig. 9C), and $30 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at Site 4 (Fig. 2B), possibly in relation to the different organic carbon contents in these 3 sediments. This suggests that organic carbon content of the sediment may determine the potential capacity of bacterial denitrification. However, the potential can be tapped only when nitrate concentration in the water column is not limiting. The implication of the high potential capacity of denitrification in the Colne estuary is that nitrate removal by denitrification could increase with nitrate loading of the estuary up to the saturation concentrations of nitrate.

In addition to stimulating the rate of denitrification, nitrate availability has also been found to influence the end-products of nitrate dissimilation by bacteria (King & Nedwell 1985), and to affect the adaptation of nitrate-reducing bacterial communities in estuarine sedi-

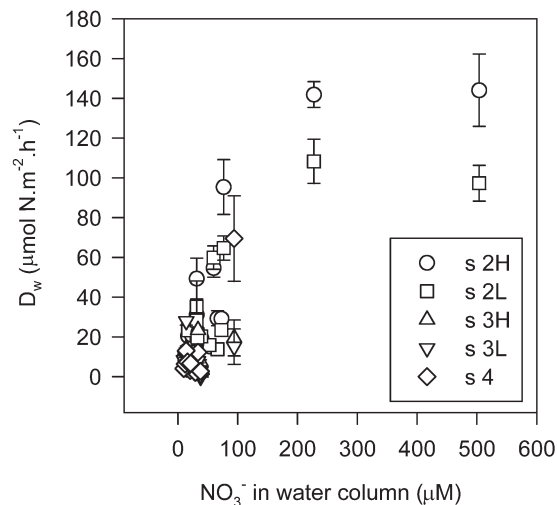


Fig. 11. Rates of D_w in darkness vs *in situ* overlying estuarine nitrate concentrations at Sites 2, 3 and 4 of River Colne estuary from October 1996 to March 1998. Bars indicate SE ($n = 5$)

ments (King & Nedwell 1987). As nitrate concentration increased, the proportion of the nitrate which was denitrified increased, while the proportion of nitrate which was reduced to ammonium correspondingly decreased (King & Nedwell 1985). Bacterial communities in those sediments having greater nitrate concentrations exhibited faster rates of nitrate reduction (King & Nedwell 1987). This adaptation probably explains the higher potential rates of denitrification by sediments at Sites 1 and 2 than at Site 4.

The half saturation concentration of nitrate ($K_m = 187.8 \mu\text{M}$) at Site 2 was not a real reflection of the affinity of denitrifying bacteria for nitrate, but an overall reflection of the 'affinity' of the sediments for nitrate. This includes overall affinity from different populations of denitrifying bacteria within the sediment, the depth of oxygen penetration in the surface layer of the sediment, the grain size of the sediment etc. Denitrifying bacteria have a high affinity for nitrate, as K_m values are commonly 10 to 50 μM (Billen 1978, Koike & Hattori 1978). A recent study (Garcia-Ruiz et al. 1998) showed that K_m increased on passing downstream from 13.1 to 90.4 μM in the Swale-Ouse river system in northeastern England.

In addition to assimilating inorganic nitrogen, the microphytobenthic biofilms also affected the rates of uncoupled and coupled denitrification. At the low shore of Site 2, the rates of D_w were significantly lower in the light (annual average $31.5 \mu\text{mol N m}^{-2} \text{h}^{-1}$) than in the dark ($44.4 \mu\text{mol N m}^{-2} \text{h}^{-1}$) ($p < 0.05$), but at other sites the rates of D_w in the light were not significantly different from those in the dark.

Among all sites in this estuary, only at Site 4 were the rates of D_n in the light (annual average $11.3 \mu\text{mol N m}^{-2} \text{h}^{-1}$) significantly higher than those in the dark ($7.8 \mu\text{mol N m}^{-2} \text{h}^{-1}$) ($p < 0.05$). However, at most sites (2L, 3H, 3L, 4) the percentages of D_n in the total denitrification ($D_w + D_n$) were significantly higher ($p < 0.05$) in the light than in the dark (annual average percentages of D_n were 56 and 34.7% in the light and dark at Site 2L; 36.3 and 16.3% at Site 3H; 38.7 and 29.4% at Site 3L; and 62.7 and 34.2% at Site 4). This suggests that oxygen production by the diatom biofilms reduced denitrification activity based on nitrate supply from the water column (D_w), probably by increasing the depth of oxic layer on the surface of the sediment and consequently increasing the diffusion path length, but stimulated coupled nitrification-denitrification (D_n). The overall effect was to increase the percentage of D_n in the total denitrification.

This conclusion was supported by the results of the short-term kinetic experiments. At Site 1, the rates of D_n were significantly stimulated by the O_2 produced by the biofilms (Fig. 9B), while at Site 2H the rates of D_w were significantly reduced by the O_2 produced by the

biofilms (Fig. 9C). At Site 1, although the artificial seawater used for the kinetic experiment was ammonium-free, the ammonium concentration in the pore water of the sediment was very high (2 to 3 mM); so for nitrification in the sediment availability of ammonium was unlikely to have been rate-limiting. In this case, any increase in O_2 concentration in the nitrification zone would increase nitrification, and consequently the activity of coupled denitrification. At Site 2H, the lower rates of D_w in the light than in the dark were most probably due to the inhibition of O_2 produced by the diatoms. Chlorophyll *a* content in the sediment and net O_2 output in the light were 1.7 times and 2 times those at Site 1, respectively. After 15 min illumination at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, the O_2 concentration in the top 20 mm of the sediment rose to 2.3 times that of the dark control (this was measured by the oxygen microelectrode mentioned in 'Materials and methods'). The penetration of O_2 further down into the sediment increased the diffusion-path length for nitrate from the water column into the denitrifying zone, thereby retarding D_w . The rates of D_n in the dark and light were not significantly different, although high rates of net oxygen output were observed in the light. This suggests that, in the light, nitrification in the sediment at Site 2H would be ammonium-limited, but no definite conclusion can be reached in this regard since the pore-water ammonium concentration was not measured.

It should be mentioned here that there were variations in the measurement of denitrification between replicates. This was mainly due to the heterogeneity of the sediment samples, the sampling procedure for slurry after incubation, and the measurement of the gas sample by the mass spectrometer. The observed variations were in fact caused by a combination of these 3 factors.

Budgets

By multiplying the measured rates of uncoupled denitrification (D_w) (using the mean rates of high and low shores at Sites 2 and 3) at each site and in each month (from April 1997 to March 1998) by the total area of sediment (which is defined as the area totally immersed at spring tide) in the sector of the river centred around that site, the total D_w for the Colne estuary was estimated to be 6.57 Mmol N per year (for each month, the equation = [dark $D_w \times 18 \text{ h} + \text{light } D_w \times 6 \text{ h}] \times \text{area} \times 365/12$). The total annual fluxes of oxidized nitrogen (TOxN) into the estuary from both the river and the inputs of the sewage-treatment works was 14.76 Mmol N in 1996, calculated by multiplying nitrate and nitrite concentrations by freshwater flow-rate and by time. Nutrient concentrations were measured weekly, and

freshwater flow-rates were measured daily by the Environment Agency of the UK (the authority organisation in the UK). Thus, uncoupled denitrification supported by nitrate from the water column in the estuary was equivalent to 44.5% of the annual flux of total oxidised nitrogen (mainly nitrate). Because the turnover of the nitrate pool in the sediment was rapid, and the sediment nitrate pool was depleted relatively quickly after exposure by the tide, a more conservative estimate of the D_w in this estuary could be made by multiplying the rates of denitrification in each sector by the area of tidally immersed sediment in that sector, which was equivalent to about 72% of the total estuarine sedimentary area (Ogilvie et al. 1997). This gave an annual estimate of uncoupled denitrification of 4.73 Mmol N yr⁻¹, which attenuated 32% of the annual flux of total oxidised nitrogen. These estimates are very close to those of a previous study (Ogilvie et al. 1997), and indicate interannual comparability in denitrification in the Colne estuary.

Although a significant amount of TOxN can be removed from the water column of the estuary only through D_w , coupled nitrification-denitrification (D_n) also plays an important role in removal of nitrate produced via nitrification in the sediment of the estuarine. The estimated amounts of nitrogen removed by D_n were obtained by multiplying the measured rates of D_n at each site in each month (from April 1997 to March 1998) by the total sediment area in each sector (for each month, equation = [dark $D_n \times 18$ h + light $D_n \times 6$ h] \times area \times 365/12). This was 2.2 Mmol N yr⁻¹, which was one-third of the amount of N removed by D_w .

The total denitrification ($D_w + D_n$, or 72% $D_w + D_n$) of 8.77 (or 6.93) Mmol N yr⁻¹ was equivalent to 25.3% (or 20%) of the total inorganic nitrogen inputs (TOxN + ammonium = 14.76 + 19.86 = 34.62 Mmol N in 1996; ammonium load was obtained by a calculation similar to that for nitrate load) into the estuary.

It is interesting that the percentage of N removed by denitrification from the total N load into an estuary varies in different estuaries. In a small, shallow estuary with a surface area of 1.86 km² (the Norsminde Fjord, Denmark, Nielsen et al. 1995), only 2 to 3% of total N load was removed from the estuary by denitrification, while in the Colne estuary with a surface area of 5.23 km², 20 to 25% of the N load was removed by denitrification. The average annual rate of denitrification was only 0.23 mol N m⁻² yr⁻¹ in the Norsminde Fjord, while it was 1.67 mol N m⁻² yr⁻¹ in the Colne estuary, 7.3 times higher than that in the Norsminde Fjord. The lower rate of denitrification in the Norsminde Fjord was probably due to lower nitrate concentration in the water column, since from June to November 1992, nitrate concentrations were virtually zero. Moreover, at lower nitrate concentration, the ratio of

nitrate reduction to ammonium to nitrate denitrified increased (King & Nedwell 1987). Another possible reason for the lower N removal capacity in the Norsminde Fjord is the short freshwater flushing time (1.5 to 13 d) and high total N load (307 t N yr⁻¹ or 21.9 Mmol N in 1992: the proportion of nitrate in the total was not shown; it is possible that ammonium would comprise a larger proportion). The low rate of denitrification and high N load gave rise to a low percentage of N removed by denitrification (2 to 3% of the total N load). Even within any one estuary, the percentage of N removal by denitrification varies monthly. For instance, attenuation of the total oxidised nitrogen load varied from 1% in the middle of winter to 56% at the height of summer in the Great Ouse estuary, another estuary on the east coast of England (Trimmer et al. 1998).

The total nitrogen assimilated by benthic diatoms was estimated to be 0.81 Mmol N yr⁻¹ by multiplying the measured rates of net O₂ production (assuming O₂:CO₂ = 1:1, C:N = 10:1 and 6 h in the light for 1 d) at each site in each month (from April 1997 to March 1998) by the exposed sediment area (28% of the total sediment area) in each sector. It accounted for 2.3% of the total nitrogen inputs (or 4.1% of the total ammonium input) from the river and the sewage-treatment works into the estuary. It should be noted that the estimation of the total N assimilated by microphytobenthos is rather approximate, considering the possibility of wide deviations in the C:N ratio and the seasonal cycle in daylength over the year.

A comparison of N denitrified by bacteria ($D_w + D_n$) with N assimilated (using net O₂ production, C:N = 10:1) by microphytobenthos on a per unit area and per day basis can be made assuming that photosynthesis of microphytobenthos occurred during the daylight emersion period, defined as 6 h d⁻¹ for Sites 1, 2H, 3H and 4, and 3 h for Sites 2L and 3L. Fig. 12 shows that at Site 1 denitrification played an important role in removing nitrogen input from the estuary; at Site 2, denitrified N and assimilated N were similar; at Sites 3 (where chlorophyll *a* content in the sediment was the highest) assimilated N was much higher than denitrified N; and at Site 4, assimilated N was higher than denitrified N. Most of the assimilated N will probably be mineralised and removed from the estuary by coupled nitrification-denitrification.

In conclusion, denitrification in this estuary played a significant role in attenuating nitrogen inputs from the river and the sewage-treatment works. Between 32 to 44% of TOxN inputs and 20 to 25% of the total inorganic nitrogen inputs (TOxN + ammonium) into the estuary were removed from the estuary before being transported into the North Sea. The nitrate concentration in the water column seems to be the primary

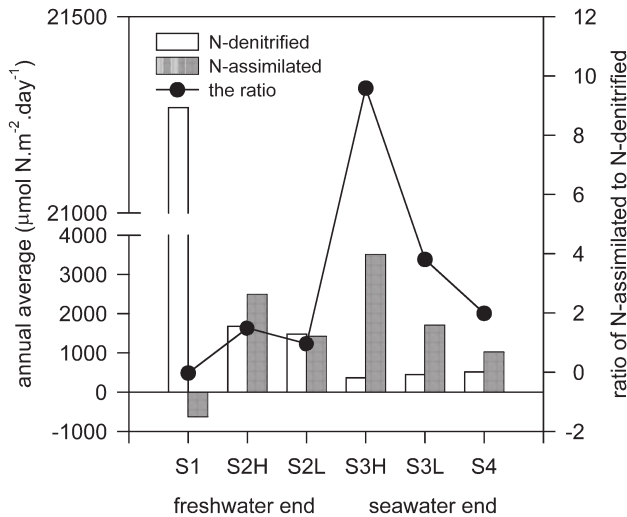


Fig. 12. Annual average of N denitrified by bacteria and N assimilated by microphytobenthos (on per unit area and per day basis) and ratio of N denitrified to N assimilated for all 6 sites in River Colne estuary

regulator of the rate of denitrification in this estuary. There were large unrealised potentials in the rates of denitrification at all sites in this estuary. Benthic primary producers, mainly diatoms, assimilated 2.3% of nitrogen input from the river and the sewage-treatment works, and temporarily retained the assimilated nitrogen in the estuary. They also affected the rates of denitrification in a complex manner. They regulated the depth of oxygen penetration by photosynthetic oxygen production and respiratory oxygen uptake. Oxygen inhibited the rates of D_w by increasing the length of the nitrate-diffusion path from the water column into the denitrifying zone (Fig. 9C), but stimulated D_n where availability of ammonium was not a limiting factor for nitrification (Fig. 9B).

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