

Sources of nitrogen used for denitrification and nitrous oxide formation in sediments of the hypernutrified Colne, the nutrified Humber, and the oligotrophic Conwy estuaries, United Kingdom

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Abstract

Rates of denitrification and nitrous oxide (N₂O) formation, and the sources of N₂ and N₂O, were examined by the isotope-pairing technique in three U.K. estuaries (Colne, Humber, Conwy), which ranged from extremely nutrified to oligotrophic. Nitrous oxide concentrations were supersaturated throughout the year with annual averages of 603% in the Colne, 158% in the Humber, and 133% in the Conwy, indicating that the estuaries were sources of atmospheric N₂O. Denitrification and N₂O formation were from benthic processes, and not from water-column processes. Generally, both denitrification and N₂O formation decreased down the estuary as nitrate concentrations lowered. The proportion of N₂ and N₂O derived from nitrate in the overlying water column (D_w) also decreased with nitrate concentration, while that from benthic coupled nitrification–denitrification (D_n) increased. Scaled to the total estuary area, in the hypernutrified Colne, water-column nitrate was the main source of N₂ and N₂O; in the moderately nutrified Humber, D_w and D_n contributed approximately equally, while in the oligotrophic Conwy, coupled nitrification–denitrification (D_n) was the main source of N₂ and N₂O. For the first time, the formation of N₂O from either the nitrification or denitrification steps of D_n was also determined. In the Colne, that from the nitrification step predominated at the top of the estuary but decreased down the estuary, while in the Humber that from denitrification dominated at the top and decreased down the estuary. In the oligotrophic Conwy, there were approximately equal contributions.

Bacterial denitrification in estuarine sediments, the reduction of nitrate to dinitrogen (N₂) and nitrous oxide (N₂O), has been identified as a process leading to nitrate removal from estuaries. Therefore the capacity of an estuary to remove anthropogenic inputs of nitrate is important in modifying the load of nitrate to coastal waters (Nedwell and Trimmer 1996; Barnes and Owens 1998; Trimmer et al. 1998; Nedwell et al. 1999; Dong et al. 2000*a,b*). Rates of denitrification vary considerably in different estuaries, at different sites within an estuary, and at different times of the year. As a result, the extent of nitrate removal by estuarine denitrification is variable from less than 1% to 100% of total nitrate input into an estuary (Nielsen et al. 1995; Ogilvie et al. 1997*a*; Barnes and Owens 1998; Trimmer et al. 1998; Dong

et al. 2000*b*). This is subject to influences of various factors that affect rates of denitrification and the bacterial community of denitrifiers in sediments (King and Nedwell 1987; Ogilvie et al. 1997*b*; Dong et al. 2000*b*), of which nitrate concentration in the water column and freshwater flushing time of an estuary seem to be dominating factors. Although the main end product of denitrification is in the form of N₂, nitrous oxide is also produced in the process of denitrification either as intermediate or as an end product or as both (Lloyd 2000; Dong et al. 2002). Other processes, such as nitrification (Goreau 1980; Jørgensen et al. 1984; Poth and Focht 1985) and nitrate reduction to ammonium (Smith and Zimmerman 1981), have also been shown to be able to produce N₂O. Information on the biological sources of N₂O is important in subsequently managing N₂O emissions. Nitrous oxide derived from nitrification may require control of point source ammonium load from sewage treatment works, while that from fluvial nitrate load may require control of diffuse nitrate sources in catchment.

Nitrous oxide is a greenhouse gas and has an atmospheric lifetime of about 150 yr and a large greenhouse warming potential (Rodhe 1990). It may also trigger reactions in the stratosphere leading to partial destruction of the ozone layer protecting the earth from biologically harmful ultraviolet radiation from the sun (Crutzen and Schmailzl 1983). Global long-term measurement series of tropospheric N₂O show an annual growth rate of about 0.25–0.31% yr⁻¹ (Weiss 1981; Khalil and Rasmussen 1992), indicating that current global sources exceed sinks (Bouwman et al. 1995). By the Kyoto

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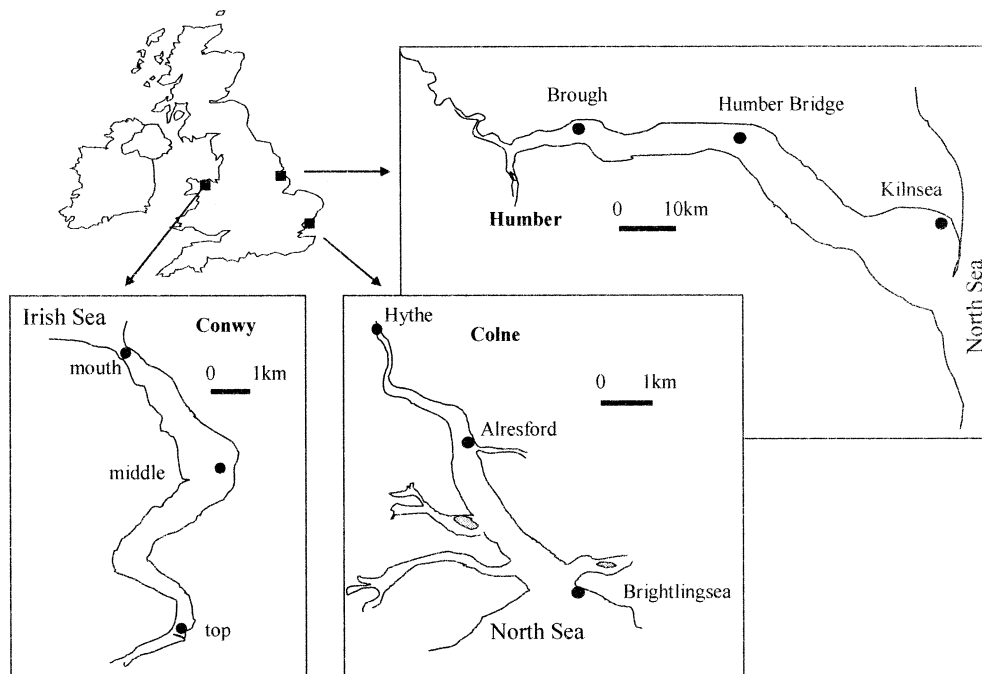


Fig. 1. Maps of the Colne, the Humber, and the Conwy estuaries showing the position of the three benthic sampling sites at top, middle, and mouth of each estuary.

Convention, the U.K. is committed to reducing its emission of greenhouse gases to 12.5% below the 1990 level by 2010. The known U.K. N_2O sources are reasonably well constrained and predominantly from industry (67 Gg) and agriculture (102 Gg) (Baggott et al. 2003). However, the aquatic source strength is much less well defined, and not even included explicitly within the latest N_2O inventory (Baggott et al. 2003). Globally, there is apparently a discrepancy between the known sources of N_2O , the rate of accumulation of N_2O in the atmosphere, and known N_2O sinks, such that there would appear to be a “missing” source of N_2O of considerable magnitude, and rivers and estuaries would account at least in part for the discrepancy (Robinson et al. 1998). Recently, Seitzinger and Kroeze (1998) concluded that rivers and estuaries could account for about 20% of the total global N_2O emissions, which is more than enough to account for the missing global source and highlights the necessity to constrain this source strength. While formation of N_2O has been reported in U.K. rivers (Garcia-Ruiz et al. 1998a,b, 1999) and estuaries (Barnes and Owens 1998; Robinson et al. 1998), their importance as sources of N_2O in the U.K. inventory is currently unknown.

In this study, three macrotidal estuaries (the Colne, the Humber, and the Conwy) with different size, nutrient load, and freshwater flushing time were chosen to investigate rates and substrates for denitrification and N_2O formation. Meanwhile, proportionation of denitrification gases (N_2O and N_2) and differentiation of N_2O from uncoupled denitrification and from coupled nitrification–denitrification and other sources were also undertaken with the aim of understanding the factors that influence emissions of nitrous oxide emission from estuaries.

Materials and methods

Sampling locations—Three estuaries were selected to cover the range from oligotrophic (Conwy), through moderately nutrient (Humber), to hypernutrient (Colne) (Fig. 1).

Colne estuary: The Colne estuary is a small, muddy, macrotidal (3- to 5-m tidal range) estuary on the east coast of England, entering the North Sea at Brightlingsea ($51^{\circ}45'N$, $01^{\circ}03'E$). The estuary catchment is 500 km², of which the River Colne drains 300 km², much of which is rich, arable land. The estuary exhibits strong gradients of both NO_3^- and NH_4^+ concentrations decreasing with distance downstream (King and Nedwell 1987; Ogilvie et al. 1997a; Robinson et al. 1998) as a result of inputs from the River Colne and a major sewage treatment work at Colchester (Nedwell et al. 2002). Three benthic sampling sites were selected (Fig. 1) at Hythe (top), Alresford (middle), and Brightlingsea (mouth), covering the full ranges of the estuarine nutrient and salinity gradients, and sediment types along the estuary.

Humber estuary: The macrotidal (5–6-m) Humber estuary is the largest estuary in the U.K., with a maximum tidal length of 147 km from Cromwell Weir on the Trent to the Humber mouth. Its catchment covers an area of ~19,127 km² (Nedwell et al. 2002), more than 20% of the land area of England (Javis et al. 1997). The freshwater input and total oxidized nitrogen input from the Humber are the largest of any British estuary to the North Sea (Nedwell et al. 2002). The catchment comprises the drainage basins of the rivers Trent and Ouse, which enter the Humber estuary at their confluence at Trent Falls. The Humber catchment is home

to 20% of the U.K. population and a very significant proportion of the energy, industrial, and agricultural production (Mortimer et al. 1998). Three benthic sampling sites were selected (Fig. 1) at Brough (top), Humber Bridge (middle), and Kilnsea (mouth).

Conwy estuary: The Conwy estuary is a small, macrotidal (3- to 5-m) estuary on the north coast of Wales, entering the Irish Sea at Conwy (53°18'N, 03°51'W). Its catchment area is 345 km² (Nedwell et al. 2002), much of which is mountainous. The Conwy estuary is a relatively oligotrophic system compared to the two east coast nutrified estuaries (Dong et al. 2000a). Three benthic sampling sites were selected (Fig. 1) at the top, middle, and mouth, covering the full ranges of the estuarine nutrient and salinity gradients, and sediment types along the estuary.

The Humber and Conwy estuaries were visited and sampled seasonally. Considerable data on denitrification and N_2O formation have already been reported for the Colne estuary (Robinson et al. 1998; Dong et al. 2000a, 2002b) and samples for measurement of denitrification and N_2O formation were only taken in this estuary during the summer when it has been shown previously that these processes were maximal. Water samples were collected at high tide and sediment samples were collected just below the low-tide mark.

Nutrient concentration in water column—Site water samples (triplicates from each site) were collected from the estuary sites and filtered through a 0.2- μ m Minisart filter (Sartorius) and frozen at -20°C for later analysis of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) on a segmented flow autoanalyser (Skalar Analytical). Salinity was measured with a refractometer (Leica).

Sediment organic carbon content—Samples of surface (0–2-cm) sediment (three replicates per site) were taken from each sampling site at low tide at the water's edge. Sediment organic carbon content was analyzed with a CHN analyzer (Model 2400; Perkin-Elmer). (See Nedwell and Trimmer 1996 for details of method used.)

Dissolved N_2O —Samples of surface water (triplicate 12.5-ml samples from each site) were taken at high tide from three sites in the estuaries with a glass hypodermic syringe and each was transferred into an exetainer (Labco Limited) containing 100 μ l formaldehyde (40% v/v) to stop any further microbiological activity. Water temperature, atmospheric pressure, and salinity were also measured as described previously (Robinson et al. 1998). On return to the laboratory, N_2O concentrations in the water samples were measured in a gas chromatograph with electron capture detector, as described in Robinson et al. (1998). The dissolved N_2O concentrations were calculated according to Weiss and Price (1980). Controls of air were always taken when sampling so that the dissolved N_2O concentrations could be calculated also as percentage saturation relative to the concentration of N_2O in air.

Measurement of rates of denitrification and N_2O production with $^{15}NO_3^-$ —The isotope-pairing technique (Nielsen

1992) was used to measure the rates of denitrification and rate of N_2O production 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 (seven replicates per site, each about 10-cm deep) were collected in perspex tubes (3.4-cm internal diameter \times 22-cm length) at low tide from each site. On returning 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 aerated site water to reequilibrate after coring. The next day, rates of denitrification and N_2O production were measured as described previously (Dong et al. 2000b). We have reported previously (Dong et al. 2000b) that illumination of sediment cores stimulated the importance of D_n compared to D_w because of increased benthic O_2 formation by microphytobenthic algae. We chose to incubate the sediment cores in the dark because the light-attenuation coefficient in these macrotidal estuaries is high, and once sediment is tidally covered there is rapid light attenuation; also benthic diatom mats migrate downwards into the sediment (Thornton et al. 2002), thereby minimizing benthic photosynthesis. Furthermore, while tidally covered the sediments are continually recharged with nitrate from the overlying water, but become nitrate-depleted on tidal exposure (Robinson et al. 1998). Any bioturbation, which is predominantly by small invertebrates, will also continue in the incubated cores, as in the field. The incubation conditions selected therefore mimic those for the low- and subtidal sediments that represent the majority in the estuaries.

Of the seven cores collected from each site, two were used for reference t_0 controls and the remaining five as replicates for incubation using in situ water and at in situ temperature in the dark. 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) and immediately frozen for later nitrate analysis. To the remaining five cores, $^{15}NO_3^-$ ($Na^{15}NO_3$, 99.3% ^{15}N ; Europa Scientific) was added into the water column and they were left for 30 min, which preliminary experiments had shown was sufficient for $^{15}NO_3^-$ to equilibrate. The added $^{15}NO_3^-$ was not less than 30% of the measured unlabeled nitrate (Dong et al. 2000b). Immediately prior to closing the core tubes with stoppers, 2 ml of column water was removed from each core for nitrate analysis. The ratio of unlabeled and labeled nitrate was calculated by the difference before and after addition of labeled nitrate. The water columns were stirred during incubation at 60 rpm with small magnetic followers (2-cm length) in the middle of water column to maintain mixing. Cores were incubated in a water bath at in situ temperature in the dark for 2 h to 4 h, depending on the incubation temperature. The dissolved O_2 in the water column never decreased below 80% of air saturation. At the end of incubation, the stopper was removed from each core tube and $ZnCl_2$ solution (0.5 ml of 50% w/v) was added to the water column to stop microbial activity, then the sediment and water column were stirred to mix. Two samples of the resultant slurry were immediately removed with a syringe from each core and transferred to 12.5-ml exetainers (Labco). A further

100 μl of ZnCl_2 solution was added to each exetainer, which was then sealed. One sample was for isotope analysis by isotope ratio mass spectrometer (IRMS) and the other for the analysis of N_2O concentration by GC.

Enrichment of water-column samples with ^{15}N labeled nitrate and ammonium—At the same time as the incubation of sediment cores, in situ water samples were incubated in bottles (250 ml) without (control) and with addition of ^{15}N -labeled nitrate or ammonium ($^{15}\text{NH}_4\text{Cl}$, 98% ^{15}N ; Sigma) to the same enrichment as to the sediment cores. The purpose was to examine whether or not denitrification and/or nitrification giving rise to N_2O occurred in the water column. After incubation, ZnCl_2 solution (1 ml of 50% w/v) was added to each bottle and water samples were taken for isotope analysis and for the measurement of N_2O concentration.

Nitrogen isotope analyses—Nitrogen was recovered from the slurry using a headspace methodology modified from Nielsen (1992). Slightly overpressurized slurry and water samples were supplied to the NERC Stable Isotope Facility in 12.5-ml exetainers (Labco). Four milliliters of water-slurry was removed with a gas-tight, high-precision syringe from each exetainer and simultaneously replaced with an equivalent volume of helium (BOC special gases; 99.9995% purity). Each vial was shaken manually for 5 min and then sonicated (Decon ultrasonic F5200b bath) for a further 5 min to ensure efficient extraction of the N_2 in the water to the headspace gas. The exetainers were allowed to stand for 1 min to allow residual slurry to drain from the septum port and to prevent blockage of the syringe needle prior to headspace sampling. The headspace gas (20 μl) was directly injected into an N_2 preparative interface (designed and built in-house) coupled to an IsoPrime IRMS (GV Instruments) via an open split. Prior to its introduction into the IRMS, the sample was dried by passing through $\text{Mg}(\text{ClO}_4)_2$ (Elemental Microanalysis), and CO_2 was removed with 0.7–1.2 mm Carbosorb (Elemental Microanalysis). Nitrous oxide was cryogenically trapped under liquid nitrogen, then thermally remobilized and passed over a copper-packed reduction furnace heated at 600°C to remove O_2 . The N_2 was then directed towards the triple collectors of the IRMS, where m/z 28, m/z 29, and m/z 30 mass ions were measured. Mass/charge ratios for the m/z 28, m/z 29, and m/z 30 nitrogen ($^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$) were then recorded for each sample at a trap current of 300 μA . Instrument stability checks were performed prior to each analyses by running a series of 10 reference pulses of N_2 (BOC special gases) until a standard deviation of fit better than 1.0×10^{-6} was achieved. Additionally, 10 consecutive injections (4 μl) of atmospheric air were analyzed prior to the analysis of actual samples. Precision of the instrument was better than 0.08‰ in all quality-control tests.

Isotope ratio analysis of N_2O using IsoPrime TraceGas IRMS system—Nitrous oxide from water-slurry samples was analyzed using modified headspace methods described for the analysis of N_2 , above. The headspace (~ 4 ml) was injected into a TraceGas® Preconcentrator coupled to an IsoPrime IRMS, whereupon the sample was directed through

a series of chemical traps to remove H_2O and CO_2 . The N_2O was then cryogenically trapped under liquid nitrogen. The N_2O was further cryofocused in a second liquid nitrogen trap prior to being introduced onto a 25-m \times 0.32-mm Poraplot Q gas chromatography column (Chrompack column; Varian). The column separates N_2O from any residual CO_2 and both enter the IRMS via an open split. The elapsed time between the first eluting CO_2 ($< 2 \times 10^{-10}$ A) peak amplitude of mass 44 and second eluting N_2O peak typically fall in the range between 60–70 s to avoid isobaric interference of the CO_2 with the calculated ^{15}N . The N_2O was directed towards the triple collectors of the isotope ratio mass spectrometer, where m/z 44, m/z 45, and m/z 46 mass ions are measured. Mass/charge ratios for the m/z 44, m/z 45, and m/z 46 N_2O ($^{44}\text{N}_2\text{O}$, $^{45}\text{N}_2\text{O}$, and $^{46}\text{N}_2\text{O}$) were then recorded for each sample. The various process rates were then calculated, and the equations used are summarized in Table 1.

Calculation of rates of denitrification—The rates of benthic denitrification of nitrate from the overlying water (D_w) or by coupled nitrification–denitrification (D_n) were calculated according to the equations of Nielsen (1992; see Table 1 and for more details see Dong et al. 2000b).

Calculation of rates of N_2O formation—Similarly, the rates of N_2O production were calculated (see Table 1) as $D_{15}(\text{N}_2\text{O}) = p(^{15}\text{N}^{14}\text{NO}) + 2p(^{15}\text{N}^{15}\text{NO})$, and $D_{14}(\text{N}_2\text{O}) = D_{15}(\text{N}_2\text{O}) \times p(^{15}\text{N}^{14}\text{NO})/2p(^{15}\text{N}^{15}\text{NO})$, where $D_{15}(\text{N}_2\text{O})$ and $D_{14}(\text{N}_2\text{O})$ are the rates of N_2O production based on $^{15}\text{NO}_3^-$ and $^{14}\text{NO}_3^-$, respectively. $p(^{15}\text{N}^{14}\text{NO})$ and $p(^{15}\text{N}^{15}\text{NO})$ represent the rates of production of the labeled N_2O species. Unlike the calculation for N_2 , whose concentration remains essentially constant throughout the incubation, the concentration of N_2O increased significantly through the incubation. This change in concentration was taken into account in the calculation of $p(^{15}\text{N}^{14}\text{NO})$ and $p(^{15}\text{N}^{15}\text{NO})$, using the N_2O concentrations measured in the gas chromatograph. The proportion of $D_{14}(\text{N}_2\text{O})$ supported by unlabeled NO_3^- from the water column, $D_w(\text{N}_2\text{O})$, was calculated as $D_w(\text{N}_2\text{O}) = D_{15}(\text{N}_2\text{O}) \times f/(1 - f)$, where f is the mole fraction of $^{14}\text{NO}_3^-$ in the water column. The proportion of $D_{14}(\text{N}_2\text{O})$ derived from sources other than $D_w(D_n(\text{N}_2\text{O}))$ was calculated by difference as $D_n(\text{N}_2\text{O}) = D_{14}(\text{N}_2\text{O}) - D_w(\text{N}_2\text{O})$. The percentage of D_w in the total denitrification or N_2O production was calculated as $D_w/D_{14} \times 100$.

Proportions of N_2O formation from nitrification or denitrification in D_n —While the overall proportion of N_2O formed from either D_w or D_n is clear, that from D_n might be formed either during nitrification or during the denitrification of the nitrate formed in the coupled nitrification–denitrification reaction. Can we distinguish between the two possible sources of N_2O in D_n ? It is reasonable to assume that the proportion of $\text{N}_2\text{O}:\text{N}_2$ formed from D_w , ($D_w(\text{N}_2\text{O}:\text{N}_2)$), is common to denitrification of any nitrate in the sediment at a particular site, and the rate of $D_n(\text{N}_2)$ formation at each site is known. Applying the $D_w(\text{N}_2\text{O}:\text{N}_2)$ ratio to this rate permits us to calculate the amount of N_2O formed during the denitrification stage of coupled nitrification–denitrification, $D_n(\text{N}_2\text{O})_d$. The difference between the total $D_n(\text{N}_2\text{O})$ and

Table 1. Explanation of abbreviations used in the text, the equivalent processes, and the equations used to determine the rates.

Abbreviation	Explanation	Equation
$D_{15}(N_2)$	Rate of denitrification supported by ¹⁵ N-labeled nitrate	$D_{15}(N_2) = p(^{15}N^{14}N) + 2p(^{15}N^{15}N)$, where $p(^{15}N^{14}N)$ and $p(^{15}N^{15}N)$ represent the rates of production of the labeled N ₂ species
$D_{14}(N_2)$	Rate of denitrification supported by nitrate	$D_{14}(N_2) = D_{15}(N_2) \times p(^{15}N^{14}N)/2p(^{15}N^{15}N)$
$D_w(N_2)$	Rate of denitrification supported by nitrate from overlying water column	$D_w(N_2) = D_{15}(N_2) \times f/(1 - f)$, where f is the mole fraction of ¹⁴ NO ₃ ⁻ in the water column
$D_n(N_2)$	Rate of coupled nitrification–denitrification in the sediments	$D_n(N_2) = D_{14}(N_2) - D_w(N_2)$
$D_{15}(N_2O)$	Rate of nitrous oxide production supported by ¹⁵ N-labeled nitrate	$D_{15}(N_2O) = p(^{15}N^{14}NO) + 2p(^{15}N^{15}NO)$, where $p(^{15}N^{14}NO)$ and $p(^{15}N^{15}NO)$ represent the rates of production of the labeled N ₂ O species
$D_{14}(N_2O)$	Rate of nitrous oxide production supported by nitrate	$D_{14}(N_2O) = D_{15}(N_2O) \times p(^{15}N^{14}NO)/2p(^{15}N^{15}NO)$
$D_w(N_2O)$	Rate of nitrous oxide production supported by nitrate from overlying water column	$D_w(N_2O) = D_{15}(N_2O) \times f/(1 - f)$, where f is the mole fraction of ¹⁴ NO ₃ ⁻ in the water column
$D_n(N_2O)$	Rate of nitrous oxide production from the coupled nitrification–denitrification in the sediments	$D_n(N_2O) = D_{14}(N_2O) - D_w(N_2O)$
$D_n(N_2O)_d$	Rate of nitrous oxide production from the denitrification stage of the coupled nitrification–denitrification	$D_n(N_2O)_d = D_n(N_2) \times D_w(N_2O:N_2)$, where $D_w(N_2O:N_2)$ is the proportion of N ₂ O:N ₂ formed from D_w
$D_n(N_2O)_n$	Rate of nitrous oxide production from the nitrification stage of the coupled nitrification–denitrification	$D_n(N_2O)_n = D_n(N_2O) - D_n(N_2O)_d$

$D_n(N_2O)_d$ gives N₂O formed during nitrification, $D_n(N_2O)_n$ (see Table 1).

Results

Characteristics of the sampling sites at the three estuaries—Salinities in the water columns were close to zero at the top of the estuaries, but at full coastal seawater salinities at the mouth (Table 2). Nitrate was the dominant inorganic nitrogen with concentrations much higher than nitrite and ammonium in the Colne and Humber. Ammonium was as

significant as nitrate in the Conwy. Among the three estuaries the highest average nutrients concentrations were in the Colne, the lowest in the Conwy, and the Humber was intermediate. Following the salinity gradients, there were decreasing gradients of nitrate in the Colne and Humber, with no consistent trend in the Conwy. Organic carbon contents in the top 2-cm sediments decreased down each estuary and were similar in the Colne and Humber, but generally lower in the Conwy. In the Colne at the top and middle of the estuary, sediments were fine silt muds but became sandy mud at the mouth. In the Humber, the sediments were fine

Table 2. Characteristics of three benthic sites at top, middle and mouth in the Colne, Humber, and Conwy estuaries. (Data represent means of four seasonal measurements ± SE; $n = 12$.)

Variable	Estuary	Sampling site		
		Top	Middle	Mouth
Nutrient in water column (μmol L ⁻¹)				
NO ₃ ⁻	Colne	483±73.9	90.2±31.5	5.5±5.5
	Humber	306.2±59.8	231.5±37.9	4.6±2.1
	Conwy	27.7±8.1	31.7±10.1	10.8±0.5
NO ₂ ⁻	Colne	22±9.5	5.3±2.1	0.6±0.6
	Humber	0.37±0.1	0.4±0.22	0.39±0.1
	Conwy	0.27±0.1	0.18±0.1	0.15±0.1
NH ₄ ⁺	Colne	60.5±8.9	31.6±8.9	15.6±3.6
	Humber	11.6±5.2	5.3±2.4	12.2±4.5
	Conwy	12.79±8.4	17.3±7.2	15.7±8.3
Water salinity	Colne	0.8±0.8	27.2±1.3	31.2±0.5
	Humber	0.0±0	5.0±2.0	32.2±0.7
	Conwy	0.7±0.6	20.7±4.4	29±1.6
Organic carbon content in sediment (% dry weight)	Colne	2.9±0.4	2.2±0.3	0.9±0.4
	Humber	2.6±0.4	2.5±0.4	2.0±0.1
	Conwy	1.8±0.1	1.45±0.1	0.2±0.1
Sediment type	Colne	Mud	Mud	Sandy mud
	Humber	Mud	Muddy sand	Mud
	Conwy	Mud	Mud	Sand

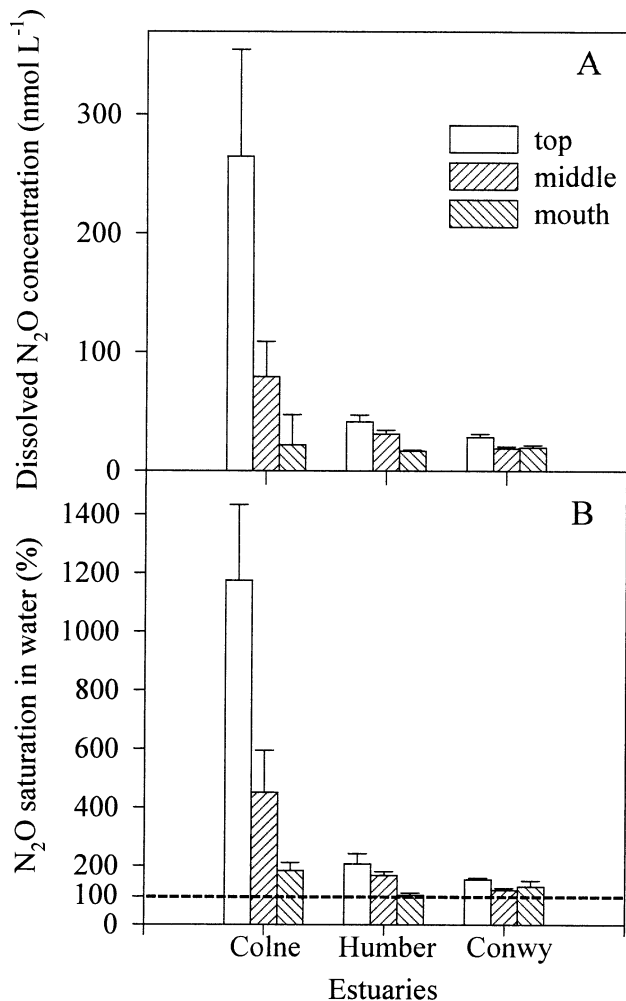


Fig. 2. Dissolved N₂O concentration and saturation (%) in the Colne, the Humber, and the Conwy estuaries. Error bars show standard error of the mean.

silts at the top and the mouth of the estuary but rather sandy in the middle. In the Conwy, there was a large mud flat in the middle and sand flat in the mouth.

In situ N₂O concentration and saturation—There was a large gradient in dissolved N₂O concentration in the water column along the Colne estuary, with concentrations being highest at Hythe (average 265 nmol L⁻¹) and decreasing down the estuary (Fig. 2A). Water column N₂O concentrations were supersaturated (Fig. 2B), averaging 1,174% of air equilibrium level at Hythe, 451% at Alresford, and 184% at Brightlingsea, indicating that the Colne estuary was a strong source of atmospheric N₂O, as reported previously (Robinson et al. 1998). Decreasing gradients of dissolved N₂O concentrations and percent saturations in the water columns were also observed down the Humber (average dissolved N₂O concentration and saturation 41 nmol L⁻¹ and 207% in the top sector at Brough, 31 nmol L⁻¹ and 167% at Humber Bridge, and 16.8 nmol L⁻¹ and 100% at Kilnsea; Fig. 2A,B). In the oligotrophic Conwy, dissolved N₂O concentrations and the saturations in the water columns were generally low-

er than those in the Colne and the Humber (averaging 28 nmol L⁻¹ and 154% at the top, 19 nmol L⁻¹ and 117% at the middle, and 19.5 nmol L⁻¹ and 129% at the mouth; Fig. 2A,B).

Rates of denitrification and N₂O production—Neither ¹⁵N-labeled N₂ nor N₂O was detected in either ¹⁵NO₃⁻ or ¹⁵NH₄⁺-enriched water-only controls but solely in ¹⁵NO₃⁻-enriched sediment cores. This confirmed that neither denitrification nor nitrification occurred in the water columns of the estuaries under air-equilibrated oxygen levels, and N₂ and N₂O were produced in the sediments and not within the water column. The water columns of all three estuaries are generally well oxygenated and unlikely to exhibit increased denitrification under low concentrations of dissolved O₂.

In the Colne estuary, rates of denitrification measured in June 2003 were 421.7, 53.7, and 8.7 μmol N m⁻² h⁻¹ at the top, middle, and bottom estuary, respectively. Rates of N₂O production were 11.3, 2.9, and 0.4 μmol N m⁻² h⁻¹ (Fig. 3A,B). Both the rate of denitrification and rate of N₂O production decreased down the estuary to the mouth, following the decreasing gradients of nitrate and nitrite concentrations (Table 2).

In the Humber, rates of denitrification and rates of N₂O production varied both seasonally and spatially. The highest rates were observed in the upper estuary in September 2001 (320 μmol N₂-N m⁻² h⁻¹ and 4.2 μmol N₂O-N m⁻² h⁻¹) and the lowest rates in December 2001 and March 2002 when temperatures were low at 0°C and 5°C, respectively. The rates of denitrification and N₂O production in mid- and bottom estuary were in the range of 0.1–39 μmol N₂-N m⁻² h⁻¹ and 0–0.54 μmol N₂O-N m⁻² h⁻¹, much lower than those at in the upper estuary (Fig. 4A,B). The trend of variation in rates of N₂O production was similar to that in rates of denitrification.

In the Conwy estuary, there were also spatial and temporal variations in the rates of denitrification and rates of N₂O production. Nitrous oxide productions were observed at all three sites, with the lowest rates at the mouth (Fig. 5A,B). The highest rate of denitrification (108 μmol N₂-N m⁻² h⁻¹) was at the middle of the estuary in August 2002. There was hardly any denitrification at the mouth of the estuary where sediment was sand.

Origins of N₂ and N₂O—The percentage of D_w(N₂) in total denitrification at the top of the Colne estuary exceeded D_n(N₂) (>90%) and decreased to the mouth of the Colne estuary—only 25% at Brightlingsea (Fig. 3C)—as reported previously (Dong et al. 2000b). In other words, uncoupled denitrification (D_w) was much higher than coupled nitrification–denitrification (D_n) at high-nitrate sites, while D_n dominated at low-nitrate sites. Similarly, the percentage of D_w(N₂O) in the total N₂O production also decreased along the Colne, being 75%, 68%, and 12% of the total at the top, middle, and lower estuary, respectively, while the percentage of D_n(N₂O) increased (Fig. 3D). In both the Humber and Conwy, there were also decreases in the percentages of D_w(N₂O) down the estuaries (Figs. 4D, 5D); but while percentage D_w(N₂) decreased down the Humber (Fig. 4C), there was no consistent trend in the Conwy (Fig. 5C). In general,

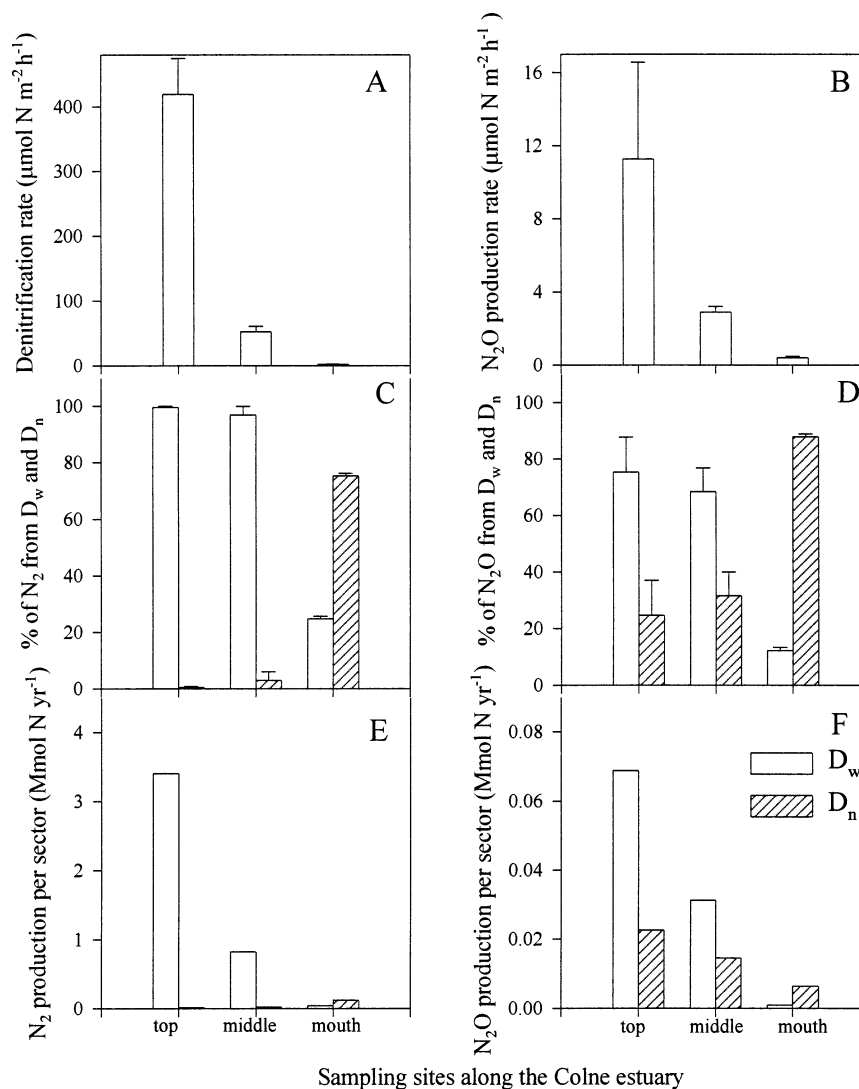


Fig. 3. (A) Rates of denitrification, (B) rates of N_2O production, (C) percentages of dinitrogen gas from D_w and D_n , (D) percentages of N_2O from D_w and D_n , (E) annual production of dinitrogen in each sector, and (F) annual production of N_2O in each sector in the Colne estuary during June 2003. Error bars show standard error of the mean.

therefore, there were increases in the percent contribution of $D_w(N_2)$ and $D_w(N_2O)$ at higher nitrate concentrations.

Of the N_2O formed in D_n , there were changes in the proportions derived from either the nitrification ($D_n(N_2O)_n$) or the denitrification ($D_n(N_2O)_d$) stages (Fig. 6). In the Colne, the higher proportion was always $D_n(N_2O)_n > 90\%$ in the top and middle but decreasing towards the mouth. In the Humber, there was the opposite trend with $D_n(N_2O)_n$ increasing towards the mouth. In the Conwy, there was no consistent trend and the greatest proportion of $D_n(N_2O)_n$ was in the middle of the estuary.

Discussion

Sources of nitrogen for denitrification and nitrous oxide formation—We have shown previously that nitrified muddy estuaries such as the Colne are sinks for nitrate (Nedwell

1975; Ogilvie et al. 1997a) and significant sources of N_2O (Barnes and Owens 1998; Robinson et al. 1998; Dong et al. 2002). Measurements along the river–estuary continuum during this present research program have shown (Dong and Nedwell unpubl. data) that N_2O concentrations and saturations usually peak in the upper reaches of estuaries, reflecting a strong N_2O source. N_2O can be formed during a number of processes including denitrification, nitrate ammonification, and nitrification, and it is currently unclear which of these processes are the predominant source of N_2O formation in estuaries.

Our data showed that N_2O concentrations were greatest in the top sectors of all three estuaries and decreased down the estuaries. The percent saturations were very high at the top of the Colne (as reported previously by Robinson et al. 1998), but much lower in the other two less nitrified estuaries. Even so, saturation values were usually greater than

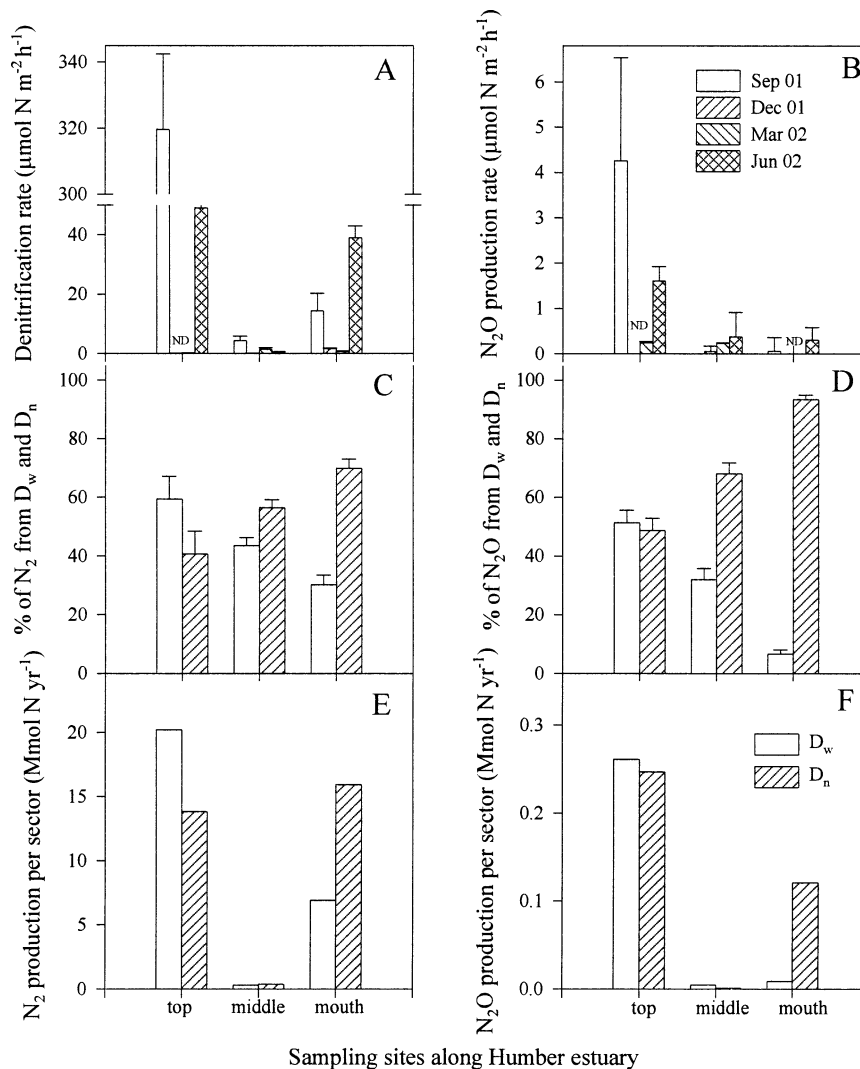


Fig. 4. Measurements at four times during the year of (A) rates of denitrification, (B) rates of N₂O production, (C) percentages of dinitrogen gas from D_w and D_n, (D) percentages of N₂O from D_w and D_n, (E) annual production of dinitrogen in each sector, and (F) annual production of N₂O in each sector in the Humber estuary. Error bars show standard error of mean. ND, not detected.

air equilibrium, and the estuaries were sources of N₂O to the atmosphere.

Our experiments showed that there was no detectable formation of N₂O in the water column, and all N₂O was formed in the bottom sediments and exchanged to the water column, as reported before for the Colne estuary (Robinson et al. 1998). The proportions of benthic denitrification and N₂O formation from water-column nitrate (D_w) varied consistently along the three estuaries. In all three estuaries, the contribution of D_w to both denitrification and N₂O formation on a unit area basis declined down the estuaries towards the mouth as the water-column nitrate concentrations declined (Figs. 3, 4, 5). In the case of the nitrified Colne estuary, during summer the proportionate contribution of D_w to both denitrification and N₂O formation was greater than in the other two less nitrified estuaries with lower nitrate concentrations.

The interpretation of our data differs in the cases of de-

nitrification and N₂O formation. In denitrification, ¹⁵N₂ is formed unequivocally from denitrification of ¹⁵NO₃⁻ added to the water above the sediment (Nielsen 1992), and the difference of this process from total denitrification must be from denitrification of unlabeled nitrate formed from benthic nitrification (D_n). Thus the trends down an estuary illustrated decreases in the rates of denitrification overall, and also a proportionate decrease in the relative importance of D_w towards D_n, i.e., increased importance of coupled nitrification–denitrification in the generally sandier sediments of lower estuaries, where nitrification has been previously reported (Ogilvie et al. 1997a).

In the case of N₂O formation, we cannot distinguish between ¹⁵N₂O formed from ¹⁵NO₃⁻ by either denitrification or nitrate ammonification, either of which process forms N₂O from nitrate as an initial substrate. In general, the ratio of nitrate denitrified:ammonified decreases with nitrate concentration (King and Nedwell 1987), and the observed trend

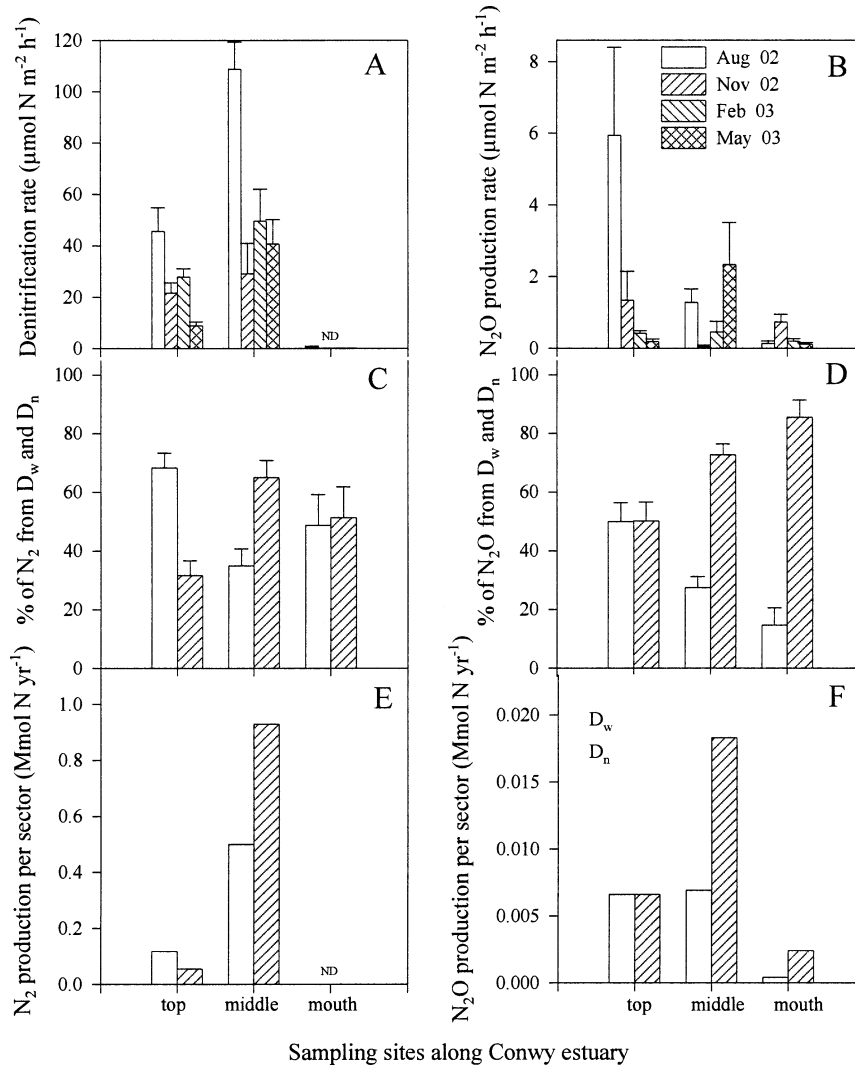


Fig. 5. Measurements at four times during the year of (A) rates of denitrification, (B) rates of N_2O production, (C) percentages of dinitrogen gas from D_w and D_n , (D) percentages of N_2O from D_w and D_n , (E) annual production of dinitrogen in each sector, (F) annual production of N_2O in each sector in the Conwy estuary. Error bars show standard error of mean. ND, not detected.

of decreased $D_w(N_2O):D_n(N_2O)$ down the estuary may also mask changes in the origins of $^{15}N_2O$ from denitrification to nitrate ammonification. However, we can distinguish N_2O formed during benthic nitrification of unlabeled NH_4^+ (or any other unlabeled substrates), which will not give labeled $^{15}N_2O$. Thus, the data indicate that in all three estuaries there were again trends of decreased benthic formation of N_2O directly from water-column nitrate ($D_w(N_2O)$), towards increased proportions of N_2O formed from benthic coupled nitrification–denitrification ($D_n(N_2O)$). At the top of the Colne estuary, during summer 75% of N_2O was derived from nitrate, but at the bottom of the estuary >80% of the albeit smaller amount of N_2O was formed from other processes including nitrification. At the top of both the Humber and Conwy estuaries, with generally lower concentrations of nitrate than in the Colne, about equal amounts of N_2O were derived from nitrate and from other substrates, but in the

middle and lower estuaries substrates other than nitrate predominated.

This was somewhat surprising in the case of the Humber estuary where, on the basis of a pilot experiment with ^{15}N -labeled nitrate or ammonium, Barnes and Owens (1998) attributed N_2O formation in the Humber estuary to nitrification in the water column, particularly in the area of the turbidity maximum. Their conclusion seems equivocal, however, as the results of experiments on benthic nitrification with sediment cores in the presence or absence of allylthiourea to inhibit nitrification, and hence measure N_2O formation by nitrification compared to uninhibited controls, were not reported and it is unclear why they attributed N_2O formation to nitrification in the water column. Our data show clearly that benthic denitrification dominated N_2O formation in the upper estuary, but changed to predominant benthic nitrification–denitrification in the lower estuary.

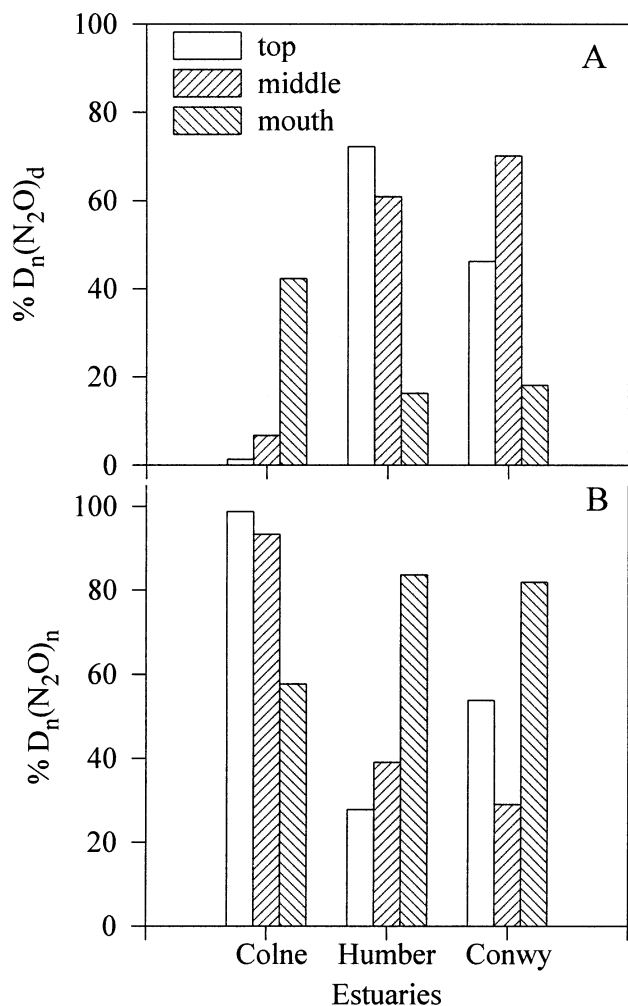


Fig. 6. Annual mean percentage of (A) $D_n(N_2O)_d$ and (B) $D_n(N_2O)_n$ in each sector along each estuary.

Relationships of mean nitrate concentrations in the water column and mean rates of denitrification and N_2O formation, and percentages of $D_w(N_2)$ and $D_w(N_2O)$ —Figure 7A shows that for the Colne and Conwy estuaries the annual mean rates of both denitrification and N_2O formation at the three sites increased with mean water-column nitrate concentrations. In contrast, the rates in the three Humber sites showed little increase with nitrate concentration. The percentages of $D_w(N_2)$ and $D_w(N_2O)$ in all three estuaries (Fig. 7B) increased with the increase of mean nitrate concentration in the water column. In the case of N_2 this indicated increased importance of uncoupled denitrification, but with N_2O it signified increased importance of either uncoupled denitrification and/or nitrate ammonification, either of which can give rise to N_2O during nitrate reduction. The shapes of the response functions are similar to rectangular hyperbolas for the data from the Colne and Conwy, but almost linear for the Humber, suggesting that the Humber sediments did not get nitrate saturated until much higher concentrations than the sediments from the other two estuaries. Secondly, in the three estuaries at any nitrate concentration the percentage of $D_w(N_2O)$ was always lower than that of $D_w(N_2)$, which sug-

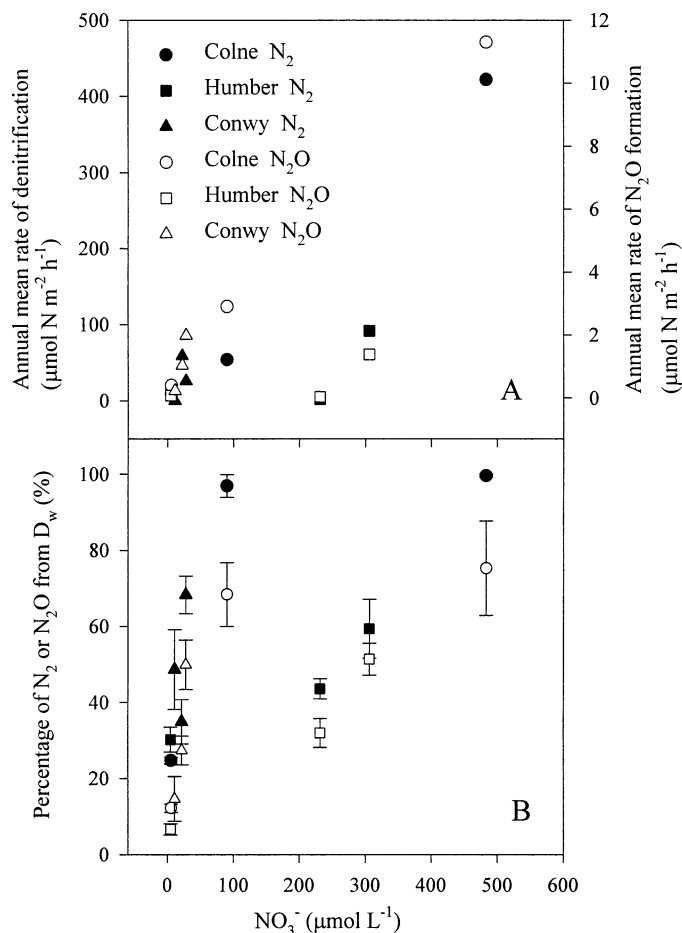


Fig. 7. Effect of annual mean nitrate concentrations in water columns on mean annual rates of denitrification and N_2O formation (A) and percentages of N_2 and N_2O produced by D_w (B) in the Colne, the Humber, and the Conwy estuaries.

gested that sources other than nitrate reduction were relatively more important in N_2O production than in N_2 production.

Calculation of total denitrification and N_2O production in each sector of the estuaries and in the whole estuaries—While rates per unit area give relative information, the overall contribution of each sector of estuary is also influenced by the area of the sector. The amount of N_2 and N_2O production from each source in each sector of an estuary was estimated by multiplying the mean seasonal rate of D_w or D_n by the area of each sector of an estuary and by time for the particular season. All seasons were then summed. In the case of the Colne estuary, this meant scaling up from the single seasonal measurements in June, when previous work had shown the majority of the N_2O was produced (Robinson et al. 1998). The annual estimate of N_2O formation obtained by scaling for the Colne estuary ($0.14 Mmol N yr^{-1}$) was very close to the $0.12 Mmol N yr^{-1}$ reported by Robinson et al. (1998) with more frequent measurements. We measured each estuary area from remote-sensing data (see Dong et al. 2004). The total area of the Colne estuary is $4.81 km^2$ (Table 3), with 19.2%, 37.5%, and 43.2% of the total area

Table 3. Total annual loads of dissolved inorganic nitrogen (DIN; means of the four years, 1995–1998), estuarine areas, calculated and measured N₂O:N₂, and nutrient effect factors for the Colne, Humber, and Conwy estuaries.

Parameter	Estuary	Data	Reference
DIN (NO ₃ ⁻ + NO ₂ ⁻ + NH ₄ ⁺) load (Mmol N yr ⁻¹)	Colne	17.7	Dong and Nedwell unpubl. data
	Humber	2,030.2	
	Conwy	27.2	
Total estuarine area (km ²)	Colne	4.8	Dong et al. 2004
	Humber	276.6	
	Conwy	5.0	
DIN load per unit area per year (×10 ⁴ kg N km ⁻² yr ⁻¹)	Colne	5.1	This study
	Humber	10.3	
	Conwy	7.7	
Predicted N ₂ O:N ₂ (%) calculated from the equation [(1.12 × 10 ⁻⁷) × kg N km ⁻² yr ⁻¹]	Colne	0.6	Seitzinger and Kroeze 1998
	Humber	1.2	
	Conwy	0.9	
N ₂ O:N ₂ (%) measured	Colne	3.3	This study
	Humber	1.1	
	Conwy	2.6	
Nutrient effect factor DIN load (kg N km ⁻² day ⁻¹) × mean FWFT (day)	Colne	1,761	This study
	Humber	12,670	
	Conwy	378	

in the sectors centered on the top (Hythe), middle (Alresford), and bottom (Brightlingsea) sectors, respectively. The total area of the Humber estuary is 277 km² (Table 3), with 15.2%, 17%, and 67.8% at the sector of Brough, Humber Bridge, and Kilnsea, respectively. The Conwy estuary has an area of 4.97 km² (Table 3), with 15.3%, 56.2%, and 28.6% of the total area at each sector from the top to the mouth (Dong et al. 2004). The total amounts of N₂ and N₂O production in each estuary were the sum of amounts from the three sectors of each estuary (Table 4).

In the Colne, the amount of N₂ produced by denitrification per sector decreased from top to bottom sectors (Fig. 3E), and uncoupled denitrification accounted for a larger proportion of the total at the top (Hythe) and middle (Alresford) (Fig. 3C). The total amount of N₂ produced in the whole estuary was 4.43 Mmol N yr⁻¹ (96.4% from *D_w* and 3.6% from *D_n*) (Table 4). The amount of N₂O production per sector decreased from top (Hythe) to bottom (Brightlingsea) (Fig. 3F). The total annual amount of N₂O produced in the whole estuary was 0.14 Mmol N yr⁻¹ (70% from *D_w* and 30% from *D_n*). Of the *D_n* component, 83% was derived from *D_n*(N₂O)_n and 17% from *D_n*(N₂O)_d. It can be concluded that the main origin of both denitrification and N₂O in the hypernutrified Colne was reduction of nitrate in the sediments, but that benthic sources other than nitrate reduction were more important for N₂O formation than for N₂ formation. The annual N₂O:N₂ ratio was 0.033 in the Colne (Table 3), i.e., only about 3.3% of the nitrogen flow to N gases was to N₂O.

In the lower nutrified conditions of the Humber estuary, the majority of N₂ and N₂O was produced in the upper estuary (Fig. 4A,B). Coupled nitrification–denitrification (*D_n*) contributed a larger proportion to the amount produced in each sector than in the Colne (Fig. 4E,F). The total amount of N₂ and N₂O produced in the whole estuary (Table 4) were 57.53 Mmol N yr⁻¹ (47.6% from *D_w* and 52.4% from *D_n*) and 0.65 Mmol N yr⁻¹ (42.0% from *D_w* and 58% from *D_n*), respectively; i.e., in the moderately nutrified Humber estuary,

approximately half of N₂O was produced from water-column nitrate (*D_w*) and the other half was from other benthic sources (*D_n*). Furthermore, of the annual N₂O from *D_n* about equal amounts were from the *D_n*(N₂O)_n and *D_n*(N₂O)_d (Table 4). The annual N₂O:N₂ ratio was 0.011 in the Humber.

In the oligotrophic Conwy estuary, the majority of N₂ and N₂O was produced in the middle sector of the estuary (Fig. 5E,F). Coupled nitrification–denitrification contributed an even larger proportion to the amount produced in each sector than in the Colne and Humber (Fig. 5C,D). The total annual amounts of N₂ and N₂O produced in the whole estuary (Table 4) were 1.604 Mmol N yr⁻¹ (38.6% from *D_w* and 61.4% from *D_n*) and 0.0412 Mmol N yr⁻¹ (33.7% from *D_w* and 66.3% from *D_n*), respectively. Thus it became very clear that in the oligotrophic Conwy estuary, benthic sources other than water-column nitrate were the main origins of N₂O, predominantly via benthic nitrification. In this estuary, as in the Humber, on a whole-estuary basis (Table 4) of the *D_n* 40% was derived from *D_n*(N₂O)_n and 27% from *D_n*(N₂O)_d. The annual N₂O:N₂ ratio was 0.026 in the Conwy.

Our data thus show that higher nitrate concentrations in an estuary result in both an increase in the mean area rates of denitrification and N₂O formation, and also in increased importance of *D_w* as a source of N₂O. In the lower-nutrient estuaries, *D_n* is a much more significant source of N₂O.

Seitzinger and Kroeze (1998) have suggested that the ratio between N₂O:N₂ fluxes is usually within the range 0.1–0.5%, although it could be as high as 6% in polluted sediments. They demonstrated that as total dissolved inorganic nitrogen (DIN; including NO₃⁻, NO₂⁻, and NH₄⁺) load increased the ratio of N₂O:N₂ increased linearly according to N₂O:N₂ = (1.12 × 10⁻⁷) × kg N km⁻² estuary yr⁻¹. The measured value in the Colne estuary (3.3%; Table 3) is, therefore, at the high end of this range, reflecting its nutrified status, but even in the less nutrified Humber estuary the ratio is 1.1%, and in the oligotrophic Conwy it is 2.6%. In the

Table 4. Annual whole-estuary budgets of origins of N_2 and N_2O in three estuaries with different nutrient loads (values in parentheses show the percentage of the total).

Estuary	N_2 (Mmol N yr ⁻¹)			N_2O (Mmol N yr ⁻¹)			Relative N_2O formation (Mmol N km ⁻² yr ⁻¹)
	Total	D_w	D_n	Total	D_w	D_n	
Colne	4.43	4.27(96.4%)	0.16(3.6%)	0.14	0.101(70.0%)	0.039(27.3%)	0.029
Humber	57.53	27.38(47.6%)	30.15(52.4%)	0.65	0.274(42.0%)	0.204(31.3%)	0.002
Conwy	1.60	0.62(38.6%)	0.98(61.4%)	0.04	0.014(33.7%)	0.016(39.6%)	0.008

Humber the measured $N_2O:N_2$ ratio and that predicted from the Seitzinger and Kroeze (1998) model were similar, but in the Colne and Conwy the measured ratios were much higher than those predicted from the model.

Although the lowest average DIN concentrations were observed in the Conwy estuary, the average N load per unit area of estuary was even higher than that in the Colne (Table 3). This was attributable to the high rainfall and water flow in the Conwy which resulted in low nitrate concentrations in the water column, short freshwater flushing times (FWFT; 0.4–3.2 d [see Dong et al. 2000a]), and reduced effect within the estuary. In contrast, the estuary with the smallest average N load per square kilometer of estuary area, the Colne, had the highest average nutrient concentrations because of relatively small freshwater flow and longer FWFT (9–16 d; see Robinson et al. 1998). The Humber has been reported (Uncles et al. 1998) to have a relatively long FWFT of 30–60 d.

Do these values for formation of N_2 and N_2O in the three estuaries reflect the average concentrations of N and/or loads of N to the estuaries? Table 4 shows the total denitrification and N_2O formation for the three estuaries, and for comparative purposes the annual rates of N_2 or N_2O formation per square kilometer of estuary area. It is clear that the Colne showed the greatest annual mean N_2 and N_2O formation rates, although this was determined from scaling from the summer rates only and should be treated with caution. In order of mean annual N_2 and N_2O formation rates, the estuaries were in the order Colne > Conwy > Humber; mean annual DIN loads per square kilometer were in the order Humber > Conwy > Colne; and mean annual DIN concentrations in the order Colne > Humber > Conwy. Therefore we have to conclude that we cannot correlate the annual denitrification or N_2O formation in the estuaries with any of these variables.

It is probable that other variables such as estuarine FWFT, or a combination of other variables, regulates these processes at the whole-estuary level. A nutrient effect factor (NEF) that takes into account both the DIN load and average FWFT can be calculated (see Table 3), and shows a very small NEF for the Conwy estuary and a large NEF for the Colne, but a very much higher NEF for the Humber because of its long FWFT. Therefore, the order of NEFs—Humber > Colne > Conwy—again does not match their order of N_2 and N_2O formations; but it must be acknowledged that the FWFT for the large Humber estuary needs further confirmation.

Do our data provide any clear strategy to reduce N_2O output from estuaries while maintaining the beneficial N sink provided by benthic denitrification? This will depend on nutrient loads, the factors (including dissolved oxygen, hydrogen sulfide concentrations, and temperature) influencing the ratios of $N_2O:N_2$ in different sectors of an estuary, and the areas of sediment in each sector, together with FWFT. While our data indicated that greatly increased nutrient loads stimulated estuarine N_2O emissions, the relative importance of other environmental factors in a general paradigm remains to be clarified.

References

- BAGGOTT, S. L., I. DAVIDSON, C. DORE, J. GOODWIN, R. MILNE, T. P. MURRELLS, M. ROSE, J. D. WATTERSON, AND B. UNDER-

- WOOD. 2003. UK Greenhouse gas inventory, 1990 to 2001: Annual report for submission under the framework convention on climate change. AEA Technology.
- BARNES, J., AND N. J. P. OWENS. 1998. Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones. *Mar. Poll. Bull.* **37**: 247–260.
- BOUWMAN, A. F., K. M. VAN DER HOEK, AND L. G. C. OLIVIER. 1995. Uncertainties in the global source distribution of nitrous oxide. *J. Geophys. Res.* **100**: 2785–2800.
- CRUTZEN, P. J., AND U. SCHMAILZL. 1983. Chemical budgets of the stratosphere. *Planet. Space Sci.* **31**: 1009–1032.
- DONG, L. F., D. B. NEDWELL, I. COLBECK, AND J. FINCH. 2004. Nitrous oxide emission from some English and Welsh Rivers and estuaries. *Water Air Soil Pollut. Focus* **4**: 127–134.
- , ———, G. J. C. UNDERWOOD, AND A. SAGE. 2000a. Environmental limitations of phytoplankton in estuaries: A report to Department of Energy, Transport and Regions, UK.
- , ———, ———, D. C. O. THORNTON, AND I. RUSMANA. 2002. Nitrous oxide formation in the Colne Estuary, England: The central role of nitrite. *Appl. Environ. Microbiol.* **8**: 1240–1249.
- , D. C. O. THORNTON, D. B. NEDWELL, AND G. J. C. UNDERWOOD. 2000b. Denitrification in sediments of the River Colne estuary, England. *Mar. Ecol. Prog. Ser.* **203**: 109–122.
- GARCIA-RUIZ, R., S. N. PATTINSON, AND B. A. WHITTON. 1998a. Denitrification and nitrous oxide production in sediments of Wiske, a lowland eutrophic river. *Sci. Total Environ.* **210/211**: 307–320.
- , ———, AND ———. 1998b. Kinetic parameters of denitrification in a river continuum. *Appl. Environ. Microbiol.* **64**: 2533–2538.
- , ———, AND ———. 1999. Nitrous oxide production in the rivers Swale-Ouse, North-East England. *Water Res.* **33**: 1231–1237.
- GOREAU, T. H. 1980. Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. *Appl. Environ. Microbiol.* **40**: 526–532.
- JAVIS, H. P., C. NEAL, AND A. J. ROBSON. 1997. The geography of the Humber catchment. *Sci. Total Environ.* **194**: 87–99.
- JØRGENSEN, K. S., H. B. JENSEN, AND J. SØRENSEN. 1984. Nitrous oxide production from nitrification and denitrification in marine sediment at low oxygen concentrations. *Can. J. Microbiol.* **30**: 1073–1078.
- KHALIL, M. A. K., AND B. A. RASMUSSEN. 1992. The global sources of nitrous oxide. *J. Geophys. Res.* **97**: 14651–14660.
- KING, D., AND D. B. NEDWELL. 1987. The adaptation of nitrate-reducing bacterial communities in estuarine sediments in response to overlying nitrate load. *FEMS Microbiol. Ecol.* **45**: 15–20.
- LLOYD, D. 2000. Temperature effects on competition, selection and physiology of estuarine nitrate-respiring bacteria. Ph.D. thesis, Univ. of Essex.
- MORTIMER R. J., M. D. KROM, P. G. WATSON, P. E. FRICKERS, J. T. DAVEY, AND R. J. CLIFTON. 1998. Sediment-water exchanges of nutrients in the intertidal zone of the Humber estuary, UK. *Mar. Poll. Bull.* **37**: 261–279.
- NEDWELL D. B., 1975. Inorganic nitrogen metabolism in an eutrophicated tropical estuary. *Water Res.* **9**: 221–231.
- , L. F. DONG, A. SAGE, AND G. J. C. UNDERWOOD. 2002. Variation of the nutrients loads to the mainland U.K. estuaries: Correlation with catchment areas, urbanization and coastal eutrophication. *Estuar. Coast. Shelf Sci.* **54**: 951–970.
- , T. D. JICKELLS, M. TRIMMER, AND R. SANDERS. 1999. Nutrient in estuaries. *Adv. Ecol. Res.* **29**: 43–92.
- , AND M. TRIMMER. 1996. Nitrogen fluxes through the upper estuary of the Great Ouse, England: The role of benthic sediments. *Mar. Ecol. Prog. Ser.* **142**: 273–286.
- NIELSEN, K., L. P. NIELSEN, AND P. RASMUSSEN. 1995. Estuarine nitrogen retention independently estimated by the denitrification rate and mass balance methods: A study of Norsminde Fjord, Denmark. *Mar. Ecol. Prog. Ser.* **119**: 275–283.
- NIELSEN, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiol. Ecol.* **86**: 357–362.
- Ogilvie, B. G., D. B. NEDWELL, R. M. HARRISON, A. ROBINSON, AND A. SAGE. 1997a. High nitrate, muddy estuaries as nitrogen sinks: The nitrogen budget of the River Colne estuary (United Kingdom). *Mar. Ecol. Prog. Ser.* **150**: 217–228.
- , M. RUTTER, AND D. B. NEDWELL. 1997b. Selection by temperature of nitrate-reducing bacteria from estuarine sediments: Species composition and competition for nitrate. *FEMS Microbiol. Ecol.* **23**: 11–22.
- POTH, M., AND D. FOCHT. 1985. ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europaea*: An examination of nitrifier denitrification. *Appl. Environ. Microbiol.* **49**: 1134–1141.
- ROBINSON, A. D., D. B. NEDWELL, R. M. HARRISON, AND B. D. OGILVIE. 1998. Hypernutrified estuaries as sources of N_2O emission to the atmosphere: The estuary of the River Colne, Essex, UK. *Mar. Ecol. Prog. Ser.* **164**: 59–71.
- RODHE, H. 1990. A comparison of the contribution of various gases to the greenhouse effect. *Science* **248**: 1217–1219.
- SEITZINGER, S. P., AND C. O. KROEZE. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. *Glob. Biogeochem. Cycles* **12**: 93–113.
- SMITH, M. S., AND K. ZIMMERMAN. 1981. Nitrous oxide production nondenitrifying soil nitrate reducers. *Soil Sci. Soc. Am. J.* **45**: 865–871.
- THORNTON, D. C. O., L. F. DONG, G. J. C. UNDERWOOD, AND D. B. NEDWELL. 2002. Factors affecting microphytobenthic biomass, species composition and production in the Colne estuary (UK). *Aquat. Microb. Ecol.* **27**: 285–300.
- TRIMMER, M., D. B. NEDWELL, D. B. SIVYER, AND S. J. MALCOLM. 1998. Nitrogen fluxes through the lower estuary of the river Great Ouse, England: The role of the bottom sediments. *Mar. Ecol. Prog. Ser.* **163**: 109–124.
- UNCLES, R. J., R. G. WOOD, J. A. STEPHENS, AND R. J. M. HOWLAND. 1998. Estuarine nutrient fluxes to the Humber coastal zone, UK, during June 1995. *Mar. Poll. Bull.* **37**: 225–233.
- WEISS, R. F. 1981. The temporal and spatial distribution of tropospheric nitrous oxide. *J. Geophys. Res.* **86**: 7185–7195.
- , AND B. A. PRICE. 1980. N_2O solubility in water and seawater. *Mar. Chem.* **8**: 347–359.

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