



Nitrous oxide production and consumption in a eutrophic coastal embayment

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Abstract

Nitrous oxide (N_2O) production and consumption rates, dissolved oxygen, ammonium, and nitrate were measured in the water column of the Bedford Basin, Nova Scotia, Canada, from June 2001 to December 2002. Ammonium oxidation rates were determined during the latter half of the study period. Nitrification was the predominant source of N_2O with production rates ranging from undetectable to $1.7 \text{ nmol N}_2\text{O l}^{-1} \text{ day}^{-1}$. N_2O production from denitrification was detected on two occasions coinciding with oxygen minima and was in the range $20 - 40 \text{ pmol N}_2\text{O l}^{-1} \text{ day}^{-1}$. N_2O consumption ($-74 \text{ pmol N}_2\text{O l}^{-1} \text{ day}^{-1}$) was seen only at the lowest recorded oxygen level of $\sim 2.5 \text{ } \mu\text{mol l}^{-1}$. The yield of N_2O from ammonium oxidation ($100 \text{ mol N}_2\text{O/mol NH}_4^+$) varied from 0.01% to 0.11% and showed a negative nonlinear relationship with dissolved oxygen in the range $27 - 290 \text{ } \mu\text{mol O}_2 \text{ l}^{-1}$. Nitrification and N_2O production rates did not appear to be related to temperature or ammonium concentration but were suppressed during periods of mixing.

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1. Introduction

Nitrous oxide (N_2O) is a major atmospheric greenhouse gas that contributes about 6% to greenhouse forcing (Prather et al., 1995). The tropospheric abundance of N_2O has increased from a preindustrial level of around 270 ppbv (Khalil et al., 2002) to a 2002 level of 318 ppbv reported for Mace Head, Ireland, by the Advanced Global Atmospheric Gases Experiment (Prinn et al., 2000). The current imbalance between

sources and sinks is estimated to be $3.9 \text{ Tg N year}^{-1}$ (IPCC, 2001).

The ocean provides a major natural source of N_2O to the atmosphere estimated at about 4 Tg year^{-1} (Nevison et al., 1995) but with a high level of uncertainty. Estuaries and coastal seas, although occupying less than 20% of the total ocean area, may account for about half of the total N_2O flux (Bange et al., 1996). Two lines of evidence suggest that the coastal margin is likely to become an increasingly important source of N_2O . First, N_2O emissions from estuaries are apparently related to nitrogen loading (Seitzinger and Kroeze, 1998). Anthropogenic nitrogen fixation is at present on a similar scale to natural terrestrial nitrogen fixation

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(Galloway et al., 1995), and the amount of fixed nitrogen transported annually from land to the coastal ocean is forecast to double over the next 50 years (Kroeze and Seitzinger, 1998). Second, oxygen depletion in subsurface water is symptomatic of eutrophication in coastal regions (Livingston, 2001), and N_2O production is enhanced at low oxygen concentrations (Yoshinari et al., 1997; de Bie et al., 2002). The further development or intensification of shelf hypoxia through increasing nutrient run-off may therefore lead to an increase in marine N_2O emissions (Naqvi et al., 2000). In order to better predict the effects of coastal eutrophication on future N_2O emissions, it is crucial to develop a clear understanding of the biological mechanisms that produce N_2O and the environmental factors that influence those mechanisms.

The major known N_2O sources in aquatic environments are the microbial pathways of autotrophic nitrification and respiratory denitrification. N_2O is a by-product of the first stage of nitrification, where ammonium is oxidised to nitrite. Although the precise mechanism is not known, the efficiency of ammonium conversion to N_2O appears to be highest at low oxygen concentrations (Goreau et al., 1980; de Bie et al., 2002). In denitrification, N_2O is an intermediate in the sequential reduction of nitrate to nitrogen gas and can potentially be produced or consumed by this pathway. Nitrification and denitrification may also be coupled by the transfer of common intermediates, e.g., nitric oxide (NO) and nitrite (NO_2^-) across oxic/suboxic boundaries (Naqvi and Noronha, 1991; Yoshinari et al., 1997) adding a further level of complexity to processes that are not well understood. The difficulties met in resolving the relative importance of nitrification and denitrification in water column N_2O production are compounded in estuarine and shallow sea environments by allochthonous inputs and sediment release (Law et al., 1992).

A stable isotope method was developed to measure potential N_2O production rates from ammonium oxidation and nitrate reduction, and also N_2O loss rates, in aquatic regions of high N_2O cycling activity (Punshon and Moore, 2004). Here, we present a series of such measurements from a eutrophic temperate coastal embayment spanning a period of 18 months. The primary objectives of this study were

(1) to determine the relative importance of ammonium oxidation (nitrification) and nitrate reduction (denitrification) to subsurface water column N_2O production, and (2) to assess the yield of N_2O from ammonium oxidation over a range of oxygen concentrations. Physical and chemical water properties were also examined to determine the major controls on N_2O production.

1.1. Study site

The Bedford Basin is a small coastal embayment with a surface area of about 17 km² forming the inner part of the Halifax Harbour network on the eastern shore of Nova Scotia, Canada (Fig. 1). The Basin has a maximum depth of 70 m and is connected to the outer harbour by a 400 m wide, 20 m deep channel (The Halifax Narrows), which effectively forms a sill. The Sackville River enters the head of the Bedford Basin and is the primary component of a total freshwater input varying between 10⁴ and 10⁷ m³ day⁻¹. The Bedford Basin can therefore be described as a small fjord-type estuary.

Halifax Harbour is surrounded by the Halifax Regional Municipality, the largest conurbation on the Atlantic coast of Canada, comprising the former cities of Halifax and Dartmouth, and the town of Bedford, and serves as a convenient natural disposal conduit for sewage. The Bedford Basin currently receives an average of over 20 million litres per day of secondary effluent from the Mill Cove wastewater treatment plant located near the mouth of the Sackville River, and additional amounts of untreated sewage are discharged into the outer harbour. Average 2002 concentrations of nitrate and phosphate in Bedford Basin seawater were 18% and 41% higher than those recorded for 1967 (Li et al., 2003), and growth in effluent discharge levels over recent decades is a likely contributing factor to this increase in nutrient loading.

During summer months, the water column is typically well stratified. The upper layer consists of warmer, fresher water usually with an estuarine circulation. The deep-water mass isolated by the sill generally remains cold (<3 °C) throughout the year and may infrequently become anoxic (Platt and Conover, 1975). Water replacement events occur

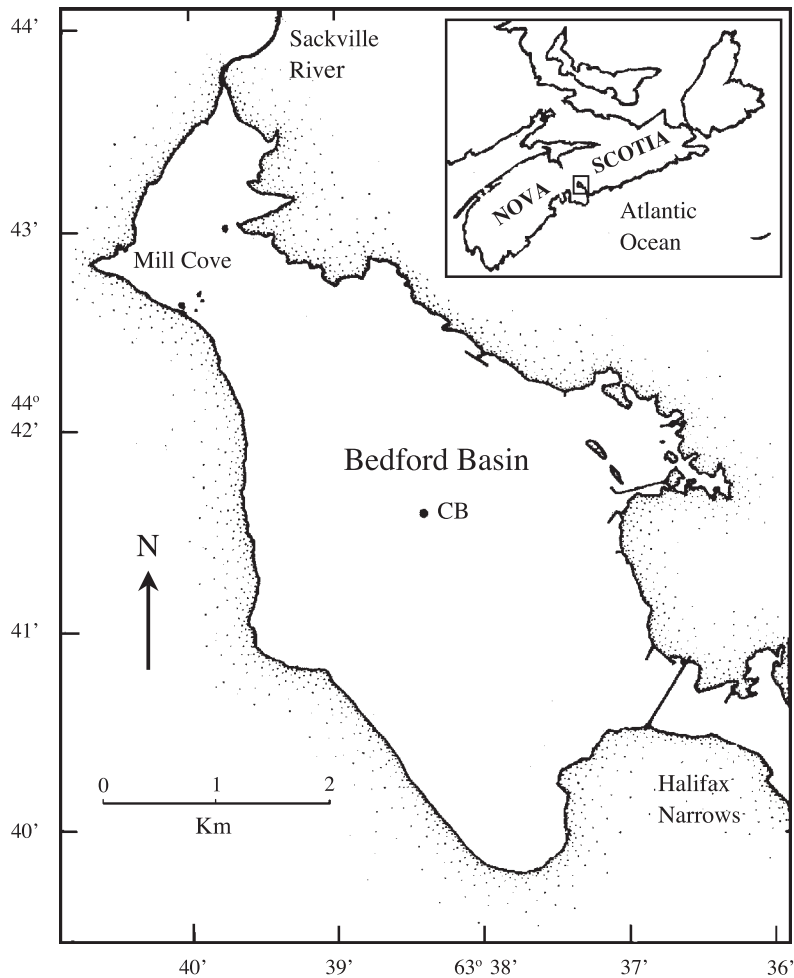


Fig. 1. A map of the Bedford Basin, Nova Scotia, Canada. CB indicates the position of the Compass Buoy Station.

irregularly but most notably during the late summer and fall (Platt et al., 1972). These events are often associated with periods of north–east winds causing a build-up in coastal sea level due to Ekman transport (Platt et al., 1972; Sandstrom, 1980; Petrie et al., 1987; Mitchell, 1990). The euphotic depth is shallow, averaging 15 m due to high turbidity (Platt et al., 1970). Tides in this region are semidiurnal with a maximum amplitude of about 2 m. All samples were collected at the Compass Buoy station ($44^{\circ}41'30''\text{N}$, $63^{\circ}38'30''\text{W}$) between 18 July 2001 and 27 November 2002. The Compass Buoy is located above the deepest point of the Basin and is the site of the Bedford Basin Plankton Monitoring

Program undertaken by the Bedford Institute of Oceanography (Li et al., 2003; Li et al., 1998). This study was coordinated with the weekly collection of conductivity and temperature data by the Bedford Institute group.

2. Methods

Water samples were collected from a small research boat using a 10 l Go-Flo sampling bottle. The bottle was lowered to the required depth by means of a cable winch equipped with a meter wheel, and valve closure was triggered by means of a messenger. Only the 60-m

depth was regularly sampled owing to the large number of analyses involved. In addition to routine 60 m sampling, additional samples were obtained from 40- and 50-m depths on September 24, October 2, and October 8th, 2002.

2.1. N_2O production and consumption rates

Duplicate water samples for measurements of N_2O production from ammonium oxidation and nitrite reduction, and also N_2O consumption, were collected in 100 ml Luer-lok glass syringes (Popper & Sons) directly from the Go-Flo sample bottle to avoid air contamination. Each syringe was filled and flushed twice before final sample collection, then sealed with a Luer-lok cap and stored in a cool box for transport.

Within 1 h of sampling, the syringes were spiked with ^{15}N -labelled substrates. In the samples used to measure N_2O production rates from ammonium oxidation or nitrate reduction, 50 μ l volumes of ^{15}N -labelled ammonium chloride or sodium nitrate solutions were added using 100 μ l Gastight[®] syringes (Hamilton, USA) to give final concentrations of 100 μ mol ^{15}N l^{-1} . In the case of samples used to measure N_2O consumption, 10 μ l of a $^{15}N_2O/N_2$ gas mixture was added to give a final concentration of ~ 1 nmol $^{15}N_2O$ l^{-1} . The sample syringes were gently shaken to ensure the substrates were well mixed, then incubated in the dark in a water bath held at within 1 $^{\circ}C$ of in situ temperature. In addition to the live samples, duplicate samples were poisoned by the addition of 0.2 ml saturated mercuric chloride solution to provide control samples.

N_2O production and consumption rates were determined by making a time series of measurements from each syringe. Subsamples were taken at intervals of about 2 h and analysed for $^{15}N_2O$ by purge-and-trap gas chromatography/mass spectrometry (P+T GC-MS). The method, which has been described in detail elsewhere (Punshon and Moore, 2004), involved purging a calibrated volume of seawater, nominally 10 ml, in a stream of UHP helium. A precisely metered volume of fully labelled internal standard gas ($^{15}N_2^{18}O$) was injected along with each sample and used to correct for variations in detector sensitivity and stripping efficiency. Moisture and CO_2 were removed from the stripped gases

by traps containing magnesium perchlorate and Ascarite. N_2O was retained in a trap consisting of a coil of stainless steel tubing immersed in liquid nitrogen. At the end of the purge cycle, the trap was heated to ~ 60 $^{\circ}C$ and the desorbed gases directed to the chromatographic columns (GS-Gas Pro, J&W Scientific) installed in a Thermo-Finnegan GC 8000 oven held at 30 $^{\circ}C$. A 15-m precolumn and a 45-m main column were configured for front-cut analysis. Once N_2O entered the main column, the accompanying suite of slower moving, less volatile gases that remained in the precolumn were back-flushed to vent. This technique eliminated the need for temperature ramping between samples and helped to prevent contaminants reaching the source and analyser components, hence improving analytical stability. Eluting gases were analysed with a Thermo-Finnegan MD800 quadrupole mass spectrometer employing electron ionisation. Ions were scanned in selected ion recording (SIR) mode, allowing a number of ions to be monitored in a repetitive, cyclic manner. Detector output was integrated using Masslab software installed on a personal computer. The NO^+ fragment ions, rather than the N_2O^+ parent ions, were measured in this study to avoid potential interference by coeluting CO_2 . The masses monitored were 30 (ambient N_2O), 31 (^{15}N -labelled N_2O), and 33 (internal standard). Analytical precision, assessed by repeated analysis of a large seawater sample held at constant temperature, was about $\pm 1\%$ [$100(\sigma/\bar{x})$]. Calibration curves were established by measuring injections of a gravimetrically prepared $^{15}N_2O$ standard gas corrected for temperature and atmospheric pressure. $^{15}N_2O$ production rates were calculated from linear regressions of normally four subsample measurements, obtained typically over a 10- to 12-h incubation period; although in some instances where production rates were very low, the incubations were extended to 24 h.

2.2. Ammonium oxidation rates

From February to December 2002, ammonium oxidation rates were measured by the chlorate block technique (Hynes and Knowles, 1983). Sodium chlorate ($NaClO_3$) inhibits the microbial oxidation of NO_2^- to NO_3^- but not the oxidation of NH_4^+ to

NO_2^- . Eight subsamples were collected in 300 ml black biological oxygen demand bottles following the method used to collect oxygen samples for Winkler analysis. In four of the bottles, 1 ml of sodium chlorate solution was added prior to stoppering to give a final ClO_3^- concentration of 10 mmol l^{-1} , the remaining four bottles were untreated control samples. The bottles were incubated at in situ temperature, and at intervals of $\sim 4 \text{ h}$, one control sample and one sample containing chlorate were analysed for nitrite using the manual colourimetric method of Hansen and Koroleff (1999). Absorbance at 540 nm was measured in a 10-cm pathlength cell with a precision of 1%. Ammonium oxidation rates were obtained from the blank-corrected increase in nitrite concentration over time. In practice, no significant change in nitrite concentration was seen in the control samples, suggesting that ammonium oxidation and nitrite oxidation rates were closely matched.

2.3. Ancillary measurements

Dissolved oxygen was routinely measured by manual Winkler titration using a Dosimat digital titrator and starch indicator with a precision of about 0.5%. On one occasion when oxygen declined to below $6 \text{ } \mu\text{mol l}^{-1}$, comparative measurements were made using a spectrophotometric method for low oxygen levels (Broenkow and Cline, 1969). A 30 ml glass syringe was filled with water from the Go-Flo bottle after flushing twice. Standard Winkler reagents (0.1 ml volumes) were added using tuberculin syringes. The resulting triiodide concentration was measured spectrophotometrically in a 1-cm cell at a wavelength of 352 nm. Measurements were calibrated with a series of potassium iodate standard solutions in the range $0\text{--}20 \text{ } \mu\text{mol l}^{-1}$ with a precision of $\pm 0.2 \text{ } \mu\text{mol l}^{-1}$.

Nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) was determined manually using a cadmium reduction column with subsequent determination of nitrite using the technique already described. Ammonium was determined spectrophotometrically using the indophenol blue method (Solórzano, 1969) with a precision of about 5%. Temperature and salinity profiles were obtained using a Seabird SBE 25-03 CTD within 2 h of sample collection.

3. Results

3.1. Measurements obtained from 60-m depth

3.1.1. Temperature, salinity, and dissolved oxygen

Figs. 2a, 2b and 2c show temporal records of temperature and salinity and dissolved oxygen. The study period was punctuated by three main mixing events resulting in perturbations to the physical and chemical water characteristics. The first event, taking place between 26 September and 17 October 2001, occurred after periods of high winds and affected only the lower water column, suggesting that this was an incursion of dense water originating from the outer harbour. In contrast, the second event was an episode of deep winter mixing during February and March 2002, when the water column attained almost uniform temperature and salinity and consequently became relatively well ventilated. The study was terminated after the third event, which took place after 25 November 2002. Between mixing events, dissolved oxygen generally showed a steady decline. From March to November 2002, salinity at 60 m remained almost constant, while temperature rose by just over $1 \text{ } ^\circ\text{C}$ in 8 months, presumably due to seasonal heating of the water column. Over the same period, dissolved oxygen showed an approximately linear decrease from $300 \text{ } \mu\text{mol l}^{-1}$ to near anoxia, consistent with biological oxygen consumption. The net rate of oxygen utilisation estimated from a linear regression of the oxygen data for this period is about $1.3 \text{ } \mu\text{mol O}_2 \text{ l}^{-1} \text{ day}^{-1}$, falling within a range of mean respiration rates ($0.5\text{--}10.1 \text{ } \mu\text{mol O}_2 \text{ l}^{-1} \text{ day}^{-1}$) reported in coastal and shelf studies (Williams, 1984; Smith et al., 1986; Iriarte et al., 1991; Pomeroy et al., 1994; Pomeroy and Peters, 1995). The lowest oxygen level observed in this study was $5 \text{ } \mu\text{mol O}_2 \text{ l}^{-1}$, measured using the Winkler method on 25 November. However, it should be noted that the sampling on this occasion was hampered by high wind and rain. A syringe sample, measured according to the method for low oxygen waters of Broenkow and Cline (1969), returned a value of $2.5 \text{ } \mu\text{mol O}_2 \text{ l}^{-1}$, and this is assumed to be the correct concentration.

3.2. Nitrate and ammonium

Fig. 3a shows the time series measurements of total $\text{NO}_3^- + \text{NO}_2^-$. The concentration ranged be-

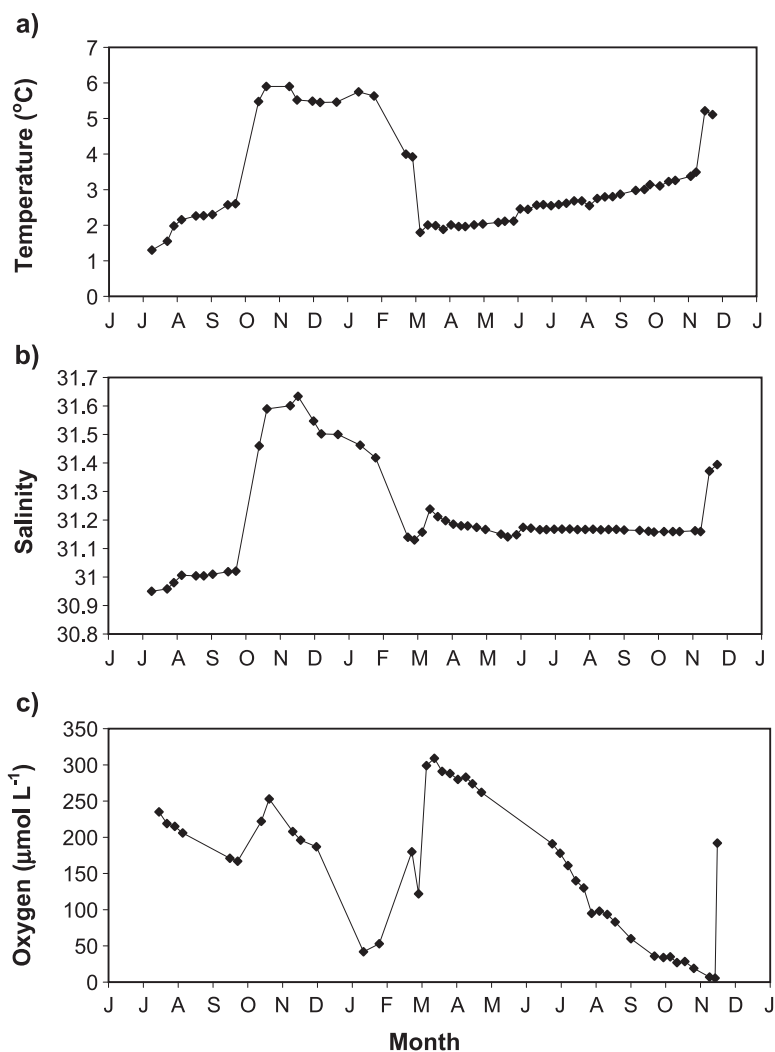


Fig. 2. Measurements of temperature (a), salinity (b), and dissolved oxygen (c) at 60-m depth in the Bedford Basin for the period June 2001 to December 2002.

tween about 5 and 20 $\mu\text{mol l}^{-1}$, with the lowest concentration being obtained during the period of deep mixing in March 2002. The record of nitrate + nitrite concentration closely mirrors that of dissolved oxygen, and there is indeed a strong negative linear correlation between these two quantities ($R^2=0.83$). This suggests that the process of nitrate supply to the deep-water mass is mainly through the oxidative decomposition of organic matter and subsequent nitrification.

In contrast, the record of ammonium displays no obvious pattern when compared with the recorded

water parameters (Fig. 3b). The main features are a large peak in August/September 2001 and a smaller peak in September/October 2002. It is not clear whether these ammonium peaks are seasonal features, but it seems unlikely, due to the timing, that they are related to periods of heightened productivity in the surface water.

3.3. N_2O production from ammonium oxidation

$^{15}N_2O$ production rates measured in samples incubated with $^{15}NH_4^+$ were generally in the range 0–

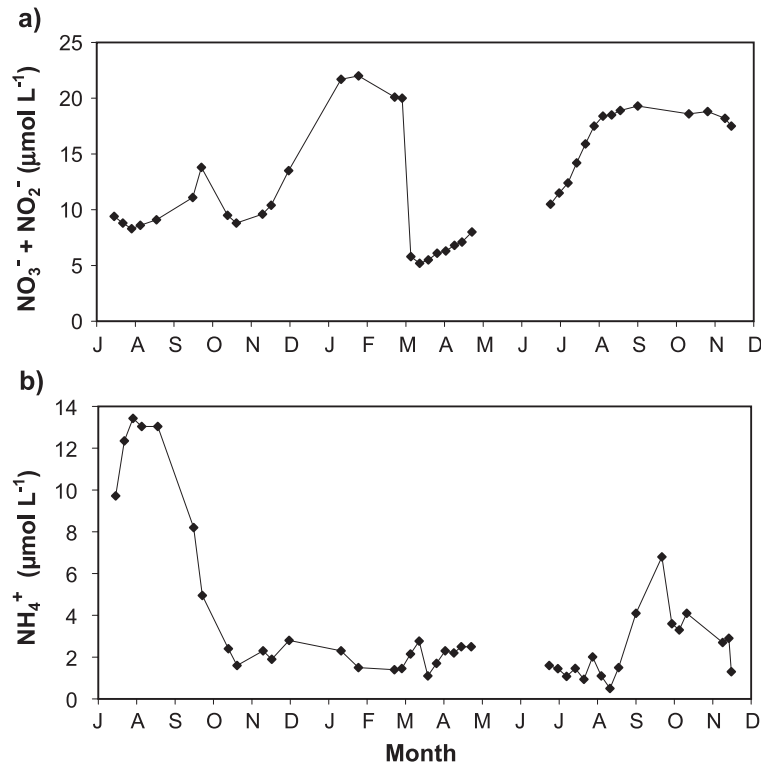


Fig. 3. Measurements of nitrate+nitrite (a) and ammonium (b) at 60 m in the Bedford Basin for the period June 2001 to December 2002.

200 $\text{pmol l}^{-1} \text{ day}^{-1}$; however, exceptionally high rates of up to $1.7 \text{ nmol l}^{-1} \text{ day}^{-1}$ were recorded in September 2001 (Fig. 4a). There was no correlation between $^{15}\text{N}_2\text{O}$ production rates and ammonium concentrations; nevertheless, the high production rates of September 2001 occurred shortly after the highest recorded level of ammonium and coincided with a steep net decline in ambient ammonium. In contrast, the second ammonium peak in September 2002 was not associated with unusually high N_2O production rates. On three occasions, coinciding with the mixing events, $^{15}\text{N}_2\text{O}$ production declined below the limits of detection ($< \sim 5 \text{ pmol N}_2\text{O l}^{-1} \text{ day}^{-1}$). The loss of production during the period of deep mixing in March 2002 was sustained for 4 consecutive weeks and was followed by a further 4 weeks of very low measurable production rates. This period of very low N_2O production was also associated with the highest levels of dissolved oxygen seen over the study period.

3.4. Ammonium oxidation rates

Ammonium oxidation rates were measured from 28 February to 27 November 2002 and ranged between 0 and about $400 \text{ nmol NH}_4^+ \text{ oxidised l}^{-1} \text{ day}^{-1}$ (Fig. 4b). There was a high degree of temporal variability, with peaks of over $350 \text{ nmol N l}^{-1} \text{ day}^{-1}$ occurring in early March, July–August, and November. Ammonium oxidation appeared to be inhibited by mixing and was undetectable for two consecutive weeks coinciding with the onset of deep mixing in winter 2002, although the subsequent recovery was rather more rapid than for N_2O production rates. The final mixing event, occurring just after 25 November 2002, suppressed the rate of N_2O production to below detection limits, but did not completely inhibit the rate of ammonium oxidation ($\sim 100 \text{ nmol N l}^{-1} \text{ day}^{-1}$). No correlation was found between ammonium concentrations and ammonium oxidation rates ($R^2 =$

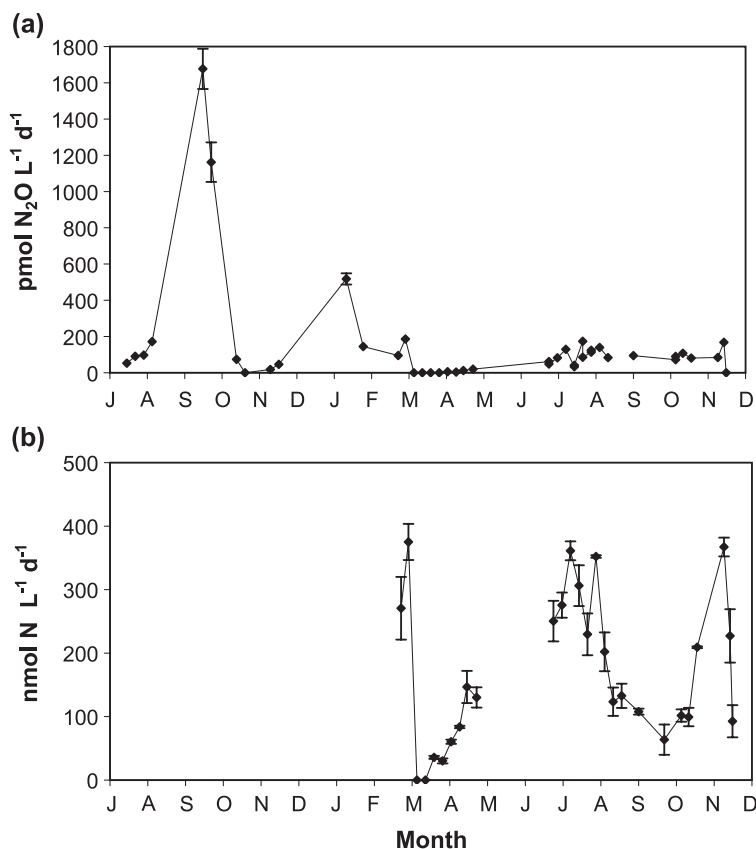


Fig. 4. (a) N_2O production rates at 60 m from samples labelled with ^{15}N -labelled ammonium for 2001 and 2002. For clarity, error bars are shown for only the three highest measurements. (b) Ammonium oxidation rates at 60 m measured in 2002. In both cases, error bars represent one standard deviation.

0.09) suggesting that this process was zero order with respect to substrate concentrations in the range $0.1\text{--}6.8 \mu\text{mol l}^{-1}$.

Although ammonium oxidation rates were not measured in 2001, the steep decline in ambient ammonium observed in September of that year, together with very high rates of N_2O production and a sharp spike in nitrate + nitrite, suggests a high rate of nitrification. In contrast, the steep increase in nitrate + nitrite from November 2001 to January 2002, together with a large rise in background N_2O , is also an evidence of high nitrification rates, yet the level of ambient ammonium remained relatively constant. This observation supports the conclusion that ammonium oxidation rates were not directly linked to substrate concentration.

3.5. N_2O production from nitrate reduction and N_2O consumption

N_2O production in samples spiked with $^{15}\text{NO}_3^-$ was seen on only two occasions. These were 16 January and 25 November 2002 with rates of 40 and 20 $\text{pmol l}^{-1} \text{day}^{-1}$, respectively. Although both instances were associated with oxygen minima, the oxygen concentrations were not comparable (42 and $2.5 \mu\text{mol l}^{-1}$). On 25 November, there was also evidence of N_2O consumption, the only instance during this study, with a mean loss rate in duplicate $^{15}\text{N}_2\text{O}$ spiked samples of $74 \text{ pmol l}^{-1} \text{day}^{-1}$. The reported production rates from ammonium oxidation and nitrate reduction for that date are therefore net values.

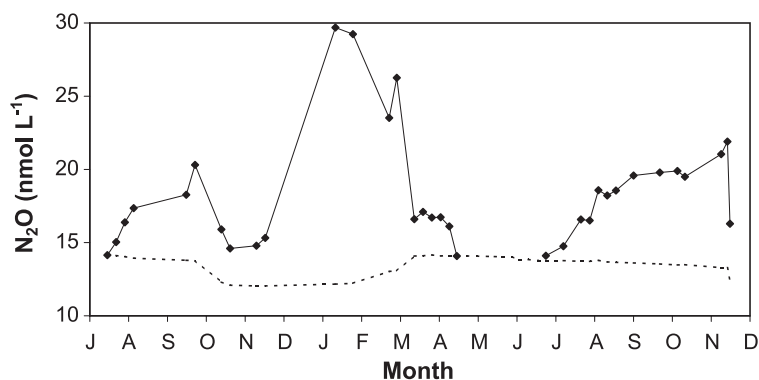


Fig. 5. The solid line represents the ambient N₂O measurements at 60 m for the period June 2001 to December 2002. The broken line represents the atmospheric equilibrium concentration of N₂O calculated from temperature and salinity data according to the equations of Weiss and Price (1980).

3.6. Ambient N₂O concentration

The concentration of background N₂O measured at 60 m, together with the atmospheric equilibrium concentration calculated from the solubility equation of N₂O in moist air (Weiss and Price, 1980) assuming a 2003 atmospheric N₂O mixing ratio of 318 ppbv, is shown in Fig. 5. The overall pattern of N₂O concentration is the reverse of dissolved oxygen, with N₂O accumulating as oxygen declines. In early July 2001, at the start of the study, N₂O concentration was close to 100% saturation. This was also the case in spring 2002, following deep mixing of the water column, and is possibly a recurring seasonal feature. During the 2 month period between November 21 2001 and January 16 2002, there is a sharp increase in ambient N₂O of almost 15 nmol l⁻¹ to a peak of 29.7 nmol l⁻¹, or 236% saturation. Although no ammonium oxidation rate data were obtained during this period, it can be concluded that there was a period

of high nitrifying activity resulting in a net average N₂O production rate of around 250 pmol l⁻¹ day⁻¹, consistent with the observed nitrate + nitrite accumulation.

3.7. Measurements from 40- and 50-m depths

Additional measurements of ammonium oxidation rates and N₂O production from labelled ammonium were made on samples collected from 40- and 50-m depths on 24 September and 2 October. There was a well-established pycnocline at ~ 25 m during this period, and temperature and salinity were nearly uniform below this depth. Extremely low levels of ammonium were evident at 40 m on both dates. The results are shown in Table 1. No production of ¹⁵N₂O was measurable in samples collected from 40, 50, and 60 m on 8 October 2002 and incubated with ¹⁵N-labelled nitrate. The oxygen concentrations in these samples ranged from 96 to 40 μmol l⁻¹.

Table 1
Results from 40- and 50-m depth obtained on 24 September and 2 October 2002

Date	Depth (m)	NH ₄ ⁺ oxidation rate (nmol N l ⁻¹ day ⁻¹)	N ₂ O production rate (pmol N ₂ O l ⁻¹ day ⁻¹)	[O ₂] (μM)	[NH ₄ ⁺] (μM)
24 September 2002	40	115 ± 16	51 ± 9	110	0.1
	50	101 ± 12	77 ± 6	69	1.9
2 October 2002	40	119 ± 17	101 ± 15	96	0.4
	50	86 ± 20	83 ± 4	56	3.0

4. Discussion

4.1. N_2O production and consumption

The ^{15}N incubation data show that in the Bedford Basin water column, nitrous oxide is derived almost exclusively from nitrification. This finding is in agreement with observations made in a number of estuarine and coastal locations including the Somali Basin (de Wilde and Helder, 1997), the Humber Estuary (Barnes and Owens, 1999), and the Schelde Estuary (de Bie et al., 2002). The two instances of measurable $^{15}N_2O$ production resulting from $^{15}NO_3^-$ reduction in 2002 coincided with oxygen minima, consistent with a process that is restricted to low-oxygen zones; however, these minima were not of comparable oxygen levels. In the first instance on 16 January, the oxygen concentration was well above the $<5 \mu\text{mol } O_2 \text{ l}^{-1}$ usually associated with denitrification zones (Codispoti et al., 2001). The circumstances facilitating denitrification at this time may have been unusual, as oxygen later declined to $19 \mu\text{mol l}^{-1}$ in November without any commensurate N_2O production in samples spiked with $^{15}NO_3^-$. There has long been speculation that denitrification may take place in anoxic microenvironments within organic particles, releasing N_2O into the relatively well-oxygenated surrounding water (Law and Owens, 1990; Ostrom et al., 2000). While such a mechanism offers an attractive explanation for the results seen on 16 January, there are no records of particulate organic matter loading that could support this explanation.

The second instance, in November 2002, of N_2O production from reduction of $^{15}NO_3^-$ is more in line with the conventional view of anaerobic denitrification. The ambient oxygen level of $\sim 2.5 \mu\text{mol } O_2 \text{ l}^{-1}$ is in the range of values associated with evidence of denitrification, such as secondary nitrite maxima (Cohen and Gordon, 1978) and isotopic enrichment of N_2O (Yoshinari et al., 1997). In this case, however, consumption of N_2O in samples incubated with $^{15}N_2O$ outweighed production, indicating that the redox conditions favoured the complete reduction of nitrogen oxides to dinitrogen. In both cases of N_2O production from labelled nitrate, similar samples spiked with labelled ammonia yielded N_2O production rates about 10 times greater. It therefore appears that denitrifica-

tion provided a very minor water column source of N_2O in the Bedford Basin during this study.

4.2. Ammonium oxidation rates

Previously reported nitrification rates in the Tamar Estuary, Chesapeake Bay, and Schelde Estuary lie in the range $3\text{--}45 \mu\text{mol N l}^{-1} \text{ day}^{-1}$ (Owens, 1986; Horrigan et al., 1990; de Bie et al., 2002). Nitrification rates in the open ocean are generally much lower, typically a few $\text{nmol N l}^{-1} \text{ day}^{-1}$ in the subsurface open ocean (Ward, 1987; Ward and Zafirou, 1988) rising to a few hundred $\text{nmol l}^{-1} \text{ day}^{-1}$ in areas of high productivity (Lipschultz et al., 1990). During the latter half of this study, nitrification rates were generally at an intermediate level between pristine ocean and highly eutrophic estuarine environments, but there was nonetheless a high degree of temporal variability. The environmental variables that affect nitrification rates have been reviewed by Ward (2000) and include temperature, light, and substrate concentration as potentially important factors. The effects of temperature and light are probably of little consequence during this study. Substantial fluctuations in nitrifying activity occurred while the temperature remained almost constant for long periods. In addition, all samples were taken from well below the euphotic zone, therefore the photoinhibition of nitrifying activity (Olson, 1981; Guerrero and Jones, 1996) should be minimal. With respect to substrate, there was no correlation between ambient ammonium concentrations and ammonium oxidation rates in this study ($R^2=0.09$), suggesting that substrate limitation was not an important factor in controlling nitrifying activity, even when water column ammonium was near the limit of detection. This finding is in agreement with zero-order kinetics for nitrification above about $0.1 \mu\text{M } NH_4^+$ reported from the field (e.g., Hashimoto et al., 1983) but contrasts with half-saturation (K_s) values in the range $0.03\text{--}3.3 \text{ mmol l}^{-1}$ reported for isolated ammonium-oxidising cultures obtained from the River Elbe (Stehr et al., 1995). One possible explanation for this observation is that most of the nitrifying biomass is particle-attached and therefore living in close association with heterotrophic bacteria. De Bie et al. (2002) for example, found that 57–86% of nitrifying bacteria in the Schelde Estuary were attached to particles.

Such association could permit regeneration of nitrate at the substrate point source, essentially decoupling nitrification from the availability of ammonium in the surrounding water.

A particularly noticeable feature of the data set was that ammonium oxidation rates and N_2O production rates were synchronously depressed during deep-water mixing. The sudden associated increase in oxygen concentration is certainly one obvious candidate for causing this effect, however another potentially important factor for which we have no information is nitrifying bacterial biomass. All other factors being equal, a change in biomass concentration linked to the introduction of a new water mass should have first-order implications for nitrification and hence N_2O production rates. Quantitative information on taxonomic microbial biomass would clearly be helpful in resolving this question.

4.3. Ambient N_2O

A plot of ΔN_2O ($[N_2O]_{\text{measured}} - [N_2O]_{\text{equilibrium}}$) against AOU, shown in Fig. 6, divides the data into two sets: those obtained prior to 23 April 02, and those obtained from 3 July to 25 November. The transition between these two data sets corresponds to a period where the concentration of ambient N_2O was near atmospheric equilibrium. For both groups of data, the relationship between ΔN_2O and AOU is

statistically best described by second order polynomial terms. In the case of the first set, the relationship ($\Delta N_2O = 0.0004\text{AOU}^2 - 0.059\text{AOU} + 4.1$) is remarkably similar to that found by Upstill-Goddard et al. (1999) for the upper 500 m of the northwestern Indian Ocean ($\Delta N_2O = 0.0004\text{AOU}^2 - 0.049\text{AOU} + 0.83$), although the latter relationship is better constrained by a much greater number of data points. The data obtained after 3 July showed a lower apparent yield of N_2O with increasing AOU ($\Delta N_2O = 0.0001\text{AOU}^2 - 0.009\text{AOU} - 0.13$). The apparent yield of N_2O with respect to oxygen consumption (mol N_2O /mol AOU) indicated by these relationships is a net value resulting from production and loss mechanisms. While there is no reason to suspect that the efficiency of the N_2O production mechanism was greater during the first half of the study, the disparity between the two curves could be explained by an increased N_2O loss rate during the latter half of 2002, reducing the apparent yield.

The period July–November 2002 is of particular interest because the relatively uniform salinity record at 60 m (Fig. 2b) suggests minimal deep-water mixing. Therefore, more so than at any other time during this study, the rate of nitrous oxide accumulation at 60 m should approximately equate to the measured production rates. Fig. 7 compares ambient N_2O data with the expected rate of N_2O accumulation for this period. The expected increase is based on linear

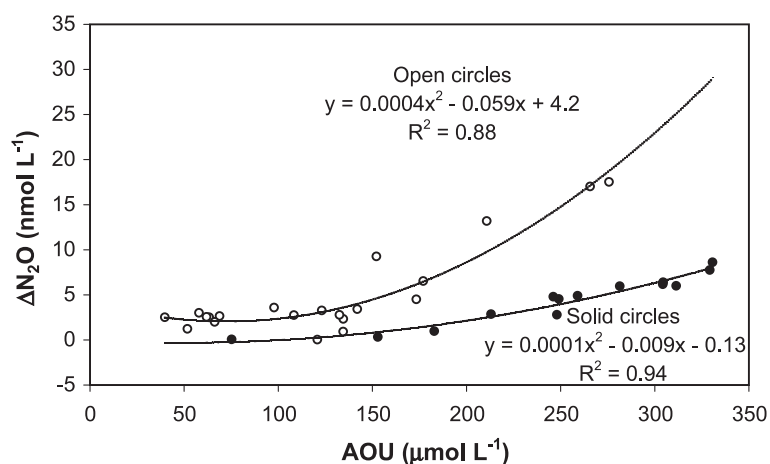


Fig. 6. ΔN_2O ($N_2O_{\text{measured}} - N_2O_{\text{equilibrium}}$) versus AOU for data collected at 60 m. The open circles represent data collected prior to May 2002, solid circles represent data collected from July to November 2002.

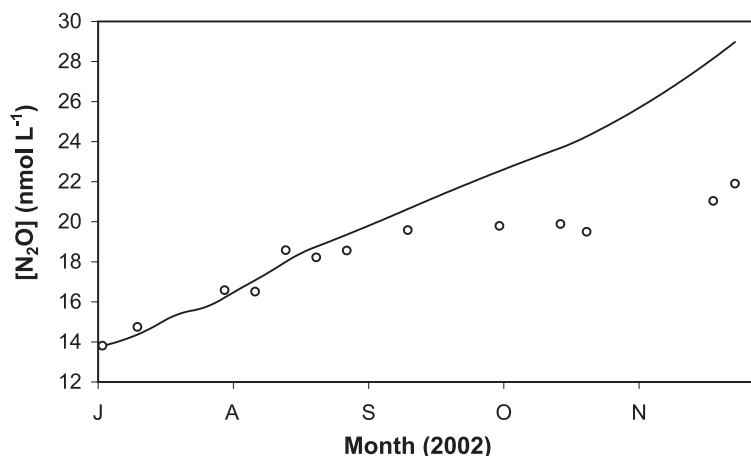


Fig. 7. A comparison of the ambient N₂O concentration data collected during July–November 2002 (open circles) with the modelled accumulation of N₂O (solid line) based on ¹⁵N₂O production rate measurements made during the same period.

interpolation of the production rate data, with the initial N₂O concentration equal to the measurement for 3 July, and assumes that there were no N₂O sinks or sources other than nitrification. Henceforth, this is referred to as the ‘modelled production’. From 3 July to 11 September, the observed accumulation rate of N₂O ($84 \pm 8 \text{ pmol l}^{-1} \text{ day}^{-1}$ from linear regression) was almost linear, in reasonable agreement with the average modelled rate of $103 \text{ pmol l}^{-1} \text{ day}^{-1}$. From the middle of September onwards, while the modelled rate of increase remained relatively constant, the observed rate of N₂O increase fell to around zero, so that little further accumulation occurred for several weeks. The nitrate + nitrite data showed a similar trend (Fig. 3a), although in that case, the inflexion occurred earlier, and there was an overall decline in concentration after the beginning of September, becoming more pronounced in November. The plateaux observed in N₂O and NO₃⁻ + NO₂⁻ concentrations, in combination with a continued decline in oxygen, suggest reductive loss of nitrogen oxides from the water column.

Although incubations did not show significant ¹⁵N₂O loss until the in situ oxygen concentration fell to about $2.5 \text{ } \mu\text{mol l}^{-1}$, it is still possible that reduction of nitrogen oxides at the sediment/water interface could provide a sink, although water column oxygen levels were above this threshold. At Compass Buoy Station, the sediment lay 10 m below the sampling depth. Moving away from this station, the water depth becomes progressively shallower with increasing dis-

tance in all directions until the 60-m isobath is reached at between 500 and 1000 m. It is therefore possible that the apparent losses of N₂O, and of NO₃⁻ + NO₂⁻, were mainly due to diffusive isopycnal and diapycnal flux to anoxic sediments, or to anoxic water overlying the sediments.

4.4. N₂O yield

If denitrification can be ignored as a source of N₂O in oxygenated water, as seems a reasonable approximation in this study, then estimates of N₂O production could be made based upon the level of nitrifying activity. Such estimates would require a knowledge of the conversion efficiency or yield of N₂O from ammonium, but as yet there are few published N₂O yields from natural populations of marine nitrifying bacteria. Indirect estimates based on observed oceanic $\Delta\text{N}_2\text{O}/\text{AOU}$ relationships combined with the Redfield model for nitrogen regeneration typically give N₂O yields of around 0.15% integrated through the water column (Elkins et al., 1978; Kaplan and Wofsy, 1985; de Wilde and Helder, 1997). de Wilde and de Bie (2000) obtained N₂O yields of 0.07–0.42% in the Schelde Estuary based on the ratio of estimated N₂O fluxes to nitrification rates, whereas in the pristine North Pacific Ocean, Yoshida et al. (1989) calculated N₂O yields in the range 0.004–0.027%, 5 to 10 times lower than some previous estimates. A plot of all calculated N₂O yields ($100 \text{ mol N}_2\text{O}/\text{mol NH}_4^+$) versus

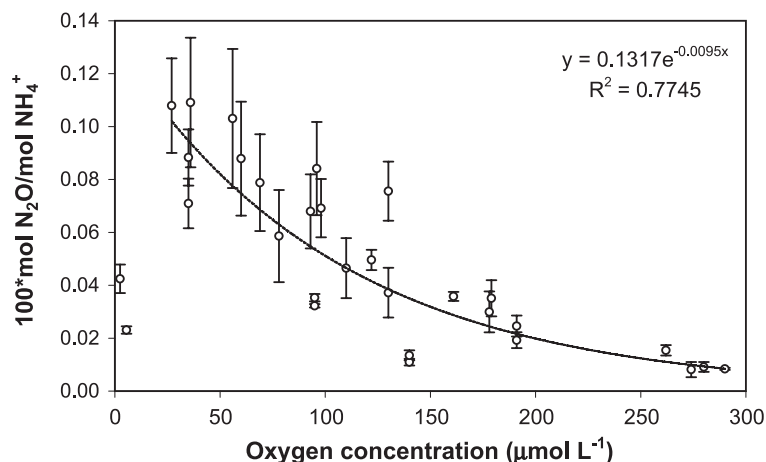


Fig. 8. Percentage N_2O yields ($100 \text{ mol N}_2\text{O/mol NH}_4^+$) for data collected between February and December 2002 showing the best-fit curve in the range $27 - 290 \mu\text{mol O}_2 \text{ l}^{-1}$. Errors are propagated from the standard deviations of N_2O production rates and ammonium oxidation rates.

oxygen concentration for the latter half of this study 2002 is shown in Fig. 8. The uncertainties are propagated from estimated errors in nitrification rates and N_2O production rates. In the range $290 - 27 \mu\text{mol O}_2 \text{ l}^{-1}$, N_2O yields vary by an order of magnitude from around 0.01% to 0.11%. There is an apparent negative nonlinear relationship, with an exponential regression giving the best statistical fit ($R^2 = 0.77$). Other studies have also reported maximal N_2O production at low oxygen concentrations (Goreau et al., 1980; de Wilde and de Bie, 2000; de Bie et al., 2002). When ambient oxygen levels were below $10 \mu\text{mol l}^{-1}$, yields were rather lower than might be expected from this relationship. Correcting the N_2O production rate obtained on 25 November 2002 for reductive removal would have a minimal effect, still leaving the N_2O yield well below those represented by the best-fit curve. This seems to point to an optimum oxygen concentration, greater than $\sim 10 \mu\text{mol l}^{-1}$ but less than $\sim 30 \mu\text{mol l}^{-1}$, where the N_2O production pathway is most efficient. An alternative possibility that must be considered is that the low apparent N_2O yields at very low oxygen levels are artifacts and result from the different sample collection methods employed for N_2O production and nitrification incubations. In the former case, where the incubation syringes are filled directly from the Go-Flo bottle after repeatedly filling and flushing, air contamination is eliminated. In the latter case, where sample collection is similar to the standard Winkler method, there is some potential for air

entrainment with the addition of the chlorate reagent and, in cases where the oxygen concentration is close to zero, invasion of oxygen across the sample surface prior to stoppering. This conclusion is supported by the differing oxygen results obtained by two analytical methods on 25 November, suggesting that the Winkler method was overestimating oxygen concentration by about $3 \mu\text{mol l}^{-1}$. Nitrification is an oxygen-requiring process, and nitrification rates can be expected to decline sharply as oxygen becomes limiting. It is therefore conceivable that nitrifying activity and hence N_2O production was oxygen limited in the syringe samples but relieved to some extent of oxygen limitation in the BOD bottles due to air contamination, hence resulting in anomalously low yield estimates. Further measurements are required to address this question.

5. Conclusions

For the period studied, ammonium oxidation was the predominant water column source of nitrous oxide in the deep part of the Bedford Basin. Denitrification was a minor source of N_2O and may constitute a net sink at very low oxygen levels. The yield of N_2O from nitrification appeared to be dependent on oxygen concentration, with suboxic water providing optimum conditions for N_2O production. There were no obvious environmental factors governing nitrification

rates; however, nitrifying activity and/or N₂O production were suppressed or much reduced during periods of mixing and sudden associated increases in dissolved oxygen. In future studies of this type, it would be helpful to evaluate ammonium oxidising bacterial biomass.

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