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Denitrification measured by a direct N_2 flux method in sediments of Waquoit Bay, MA

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Abstract. Denitrification was directly estimated in estuarine sediments of Waquoit Bay, Cape Cod, MA by detection of N_2 increases above ambient in the water overlying sediment cores. Denitrification rates (−9 to 712 μmol N_2 m^{-2} h^{-1}) were high compared to previous studies, but compared well with estimates of N loss from mass balance studies. The precision of the estimate depended on the N_2/O_2 flux ratio. The N_2/O_2 flux ratio was lower in Waquoit Bay than previously studied estuaries, and estuaries had lower N_2/O_2 flux ratios than shelf sites. The contribution of temperature-driven solubility changes to estuarine fluxes was estimated by modeling sediment temperature variations and found to be potentially important (43 μmol N_2 m^{-2} h^{-1}); however, control incubations indicate the temperature model overestimates solubility driven fluxes. The relatively low fluxes under anaerobic conditions and the low rate of NO_3^-/NO_2^- removal from the overlying water indicates coupled nitrification/denitrification produced the observed N_2 fluxes.

Key words: denitrification, estuary, method, microbial, sediments, temperate

Introduction

Denitrifying bacteria use nitrate (NO_3^-) and nitrite (NO_2^-) as electron acceptors in the oxidation of organic matter, and produce nitrous oxide (N_2O) and dinitrogen (N_2) as end products. NO_3^- and NO_2^- denitrified in sediments can originate from diffusion from overlying water or from nitrification in the sediments (Jenkins & Kemp 1984). In estuaries denitrification may remove 40–50% of the nitrogen entering the system (Seitzinger 1988), and up to 75% of the organic nitrogen mineralized in sediments (Seitzinger 1988; Kemp et al. 1990). Given the importance of nitrogen in controlling coastal productivity (Howarth 1988), it is important to quantify denitrification.

Because of the difficulty in detecting the release of N_2 against high background concentrations, most researchers have indirectly inferred rates based on other parameters. Denitrification has been estimated in estuarine sediments indirectly by acetylene inhibition (Sørensen 1978) and labelled nitrogen tracers (Nishio et al. 1982; Rysgaard et al. 1993). Both these methods can underestimate coupled nitrification/denitrification (Seitzinger et al. 1993). Acetylene inhibits nitrification (Hynes & Knowles 1978), thus causing

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an inhibition of coupled nitrification/denitrification (Seitzinger et al. 1993). $^{15}N_2$ produced after $^{15}NO_3$ addition does not include denitrification of nitrate produced in sediments (Seitzinger 1988). To account for coupled nitrification/denitrification Binnerup et al. (1992) and Nielsen (1992) modified acetylene inhibition and ^{15}N tracer techniques respectively and Seitzinger et al. (1980) and Devol (1991) developed techniques that measure N_2 flux directly.

Direct flux measurements require minimal manipulations to the nitrogen cycle. Seitzinger et al. (1980) measured denitrification directly by lowering the background N_2 concentration and measuring flux of N_2 and N_2O from sediments into a low N_2 headspace. To reduce background N_2 levels the overlying water was replaced with N_2-free water several times over the course of at least a week. This long preincubation could impart artifacts. Nowicki (1994) reduced preincubation time by using anaerobic incubations to determine non-denitrification N_2 flux. Anaerobic cores were used to eliminate coupled nitrification/denitrification and, after the exhaustion of the nitrate pool, allow estimation of the N_2 flux produced by physical processes. Anaerobic controls, however, may inhibit bioturbation and thus could underestimate bioturbation-facilitated flux (Nowicki 1994). Furthermore, because the core section must be transferred to a glass gas tight system, the procedure is difficult to adapt to *in situ* measurements.

Devol (1991) estimated denitrification by detecting changes from ambient in the N_2 concentration of water above shelf sediments. Because N_2 concentrations vary little from atmospheric solubility over the course of incubations, Devol's method minimizes risk of atmospheric contamination, and allows *in situ* measurements, but it does require a precise means of measuring dissolved gases. The flux of N_2 across the sediment water interface is determined by placing a benthic chamber on sediments and measuring the accumulation of N_2. To date this method has only been applied to upwelling sites where high nitrate concentrations create significant NO_3^- uptake rates relative to sediment metabolism.

NO_3^-, NO_2^-, and O_2 pools are depleted during chamber and core incubations (Nielsen 1992). This depletion can inhibit sediment denitrification, and could lead to underestimation of rates (Nowicki 1994). Availability of oxygen for sediment nitrifiers is particularly important because of the importance of coupled nitrification/denitrification in coastal sediments (Jenkins & Kemp 1984). Estuarine sediments may have higher denitrification rates than shelf sites, but if there is a lower N_2/O_2, less N_2 change from ambient concentration will occur before O_2 exhaustion. Even with a precise means of measuring N_2 changes, the error in the detection of small changes could result in low precision in flux measurements.

In estuaries, other processes besides denitrification could produce N_2 flux. Changes in temperature could produce N_2 fluxes because temperature changes the solubility of N_2 in sediment porewater. Sediments take up N_2 as temperatures decrease in the fall, and this stored N_2 is released as sediments warm up in the spring. Net non-denitrification N_2 flux could also occur by gas ebullition. CH_4 bubbles produced in sediments can strip N_2 from porewaters (Kipphut & Martens 1982). This bubble formation, which peaks in the summer (Chanton et al. 1989), could deplete sediment N_2 pools and create a net diffusive flux into the sediments.

In this paper the direct technique of Devol (1991) was applied to estimate denitrification in cores of estuarine sediments incubated in the lab. The procedure of Grundmanis (1989) was modified to precisely measure dissolved gases in seawater. The relative importance of non-denitrification N_2 fluxes was also assessed by using anaerobic controls to inhibit coupled nitrification/denitrification and by measuring sediment NO_3^-/NO_2^- uptake to determine denitrification of NO_3^- and NO_2^- from overlying water. The potential contribution of temperature changes to N_2 flux was calculated by modelling porewater temperature and subsequent solubility changes.

Materials and methods

Study site

Denitrification was measured in Waquoit Bay, Cape Cod, MA. Waquoit Bay is the site of the Waquoit Bay Land Margin Ecosystem Research project (Valiela et al. 1992). Denitrification was measured in three estuaries of the system: Childs River, Quashnet River, and Sage Lot Pond (Fig. 1). In the Childs River, where most of the flux measurements were made, water column salinities at the surface increase down estuary and range from 25 ppt at the surface to 30 ppt at depth in the lower reach of the estuary (LaMontagne 1996). The sediments in these estuaries are fine-textured and organic-rich. Porosity of sediments ranged from 0.88 at the surface to 0.83 at 20 cm (LaMontagne 1996). Percent carbon of sediments ranged from 6.6 at the surface to 4.8 at 20 cm (K. Foreman and K. Gribble unpubl. data).

Sampling

A site was selected for core sampling in the middle of each of the estuaries (Fig. 1). Replicate cores were taken within a 5 m radius of these sites. Divers collected sediment cores in 15.5 cm (I.D.) by 45 cm PVC tubes (Dornblaser et al. 1989). Approximately 30 cm of mud was collected in each core, leaving

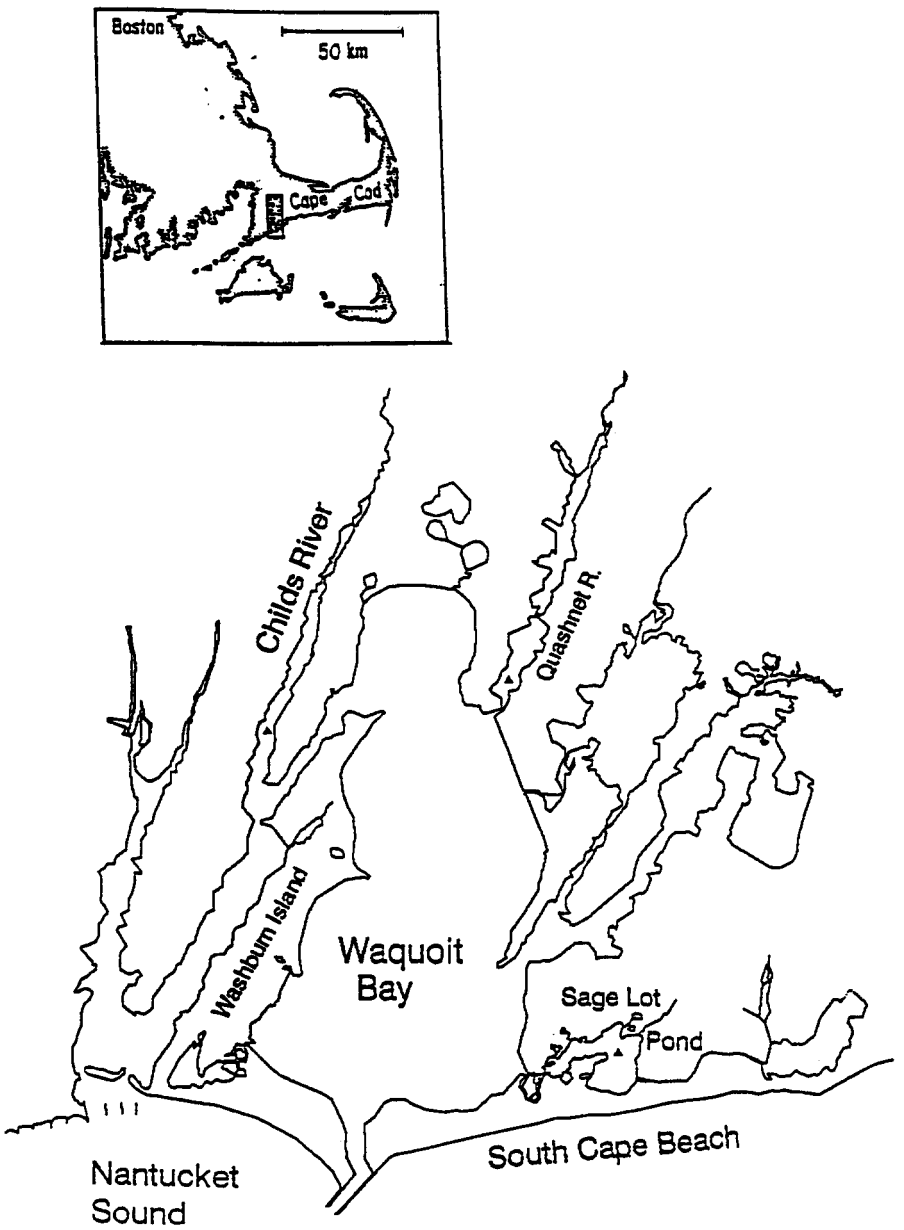

Fig. 1. Waquoit Bay, Cape Cod MA. Triangles indicate sediment sampling sites. Inset shows location of Waquoit Bay on Cape Cod and relative to Boston MA.

a headspace volume of 31. The cores were capped, packed in ice and immediately transported to the lab. Care was taken not to disturb the sediment water interface. In the lab, we submerged the cores in a sea table tank and removed the caps. Running sea water continuously replaced the tank water. A small aquarium pump aerated the water above the core until the start of the incubation. The water temperature in the lab did not differ significantly from *in situ* temperatures except in late summer when field temperatures were several degrees higher than the lab temperatures (LaMontagne 1996). The cores were allowed to equilibrate for at least 24 hr before sampling.

Flux measurements

Denitrification was estimated by measuring time courses of N_2 flux into water overlying sediments. Flux was estimated as the slope of the concentration versus time curve. 50 ml nylon syringes (S.E.S.I. France) were readied for sampling by washing in a 10% HCL solution for 10 min and filling, successively, with deionized water and seawater. The nylon syringes were tested against other plastic syringes and glass/teflon syringes and found to be gas tight (LaMontagne 1996). Air bubbles in the syringes were carefully removed. At the start of flux measurements the aerators were removed and a Plexiglass top was fitted to the cores. The tops contained 2 syringe ports for sampling and a stir bar to mix the headspace (Dornblaser et al. 1989).

Because of the importance of NO_3^- availability in controlling direct denitrification, NO_3^- concentrations at the start of the incubation were matched to *in situ* levels. NO_3^- concentration in the headspace was matched to *in situ* levels by injecting potassium nitrate dissolved in seawater.

Syringe samples were collected for gas and nutrient analysis over the course of the flux measurements. The length of the incubation depended on the sediment O_2 consumption rate. Flux measurements were run until oxygen in the headspace was exhausted. Incubations in the summer were generally run for less than 4 h, whereas in the winter incubations ran overnight. Time points from the linear range of O_2 consumption (generally until 20% of initial O_2 concentrations remained) were used for O_2 flux measurements. Water removed by syringe sampling was replaced with sea table water by venting the core to the surrounding water during sampling. The syringes were stored in ice water until the incubation was complete, and the gas concentrations in the syringes determined within 48 hr of sampling.

Dissolved gas analysis

Concentrations of O_2 and N_2 were determined with a custom gas stripper connected to a gas chromatograph. This system was designed to precisely

measure dissolved gases by injecting a fixed volume of liquid, completely stripping the gases from the sample into the carrier gas of a gas chromatograph and separating N_2 and O_2 into discrete peaks. This system uses a 10 and a 4 port gas sampling valve, an electric actuator and a digital controller (Valco Instr., Houston, TX) to inject a liquid sample into a stripping chamber (Fig. 2). The stripping chamber (Glassblowers Inc., Turnersville, NJ) is a glass tube with a ceramic frit in the bottom. Carrier gas (ultrapure helium) flowing through this frit bubbles the liquid sample and strips dissolved gasses into the gas flow. Two MS-5A columns, a 1.8 m precolumn and a 3.1 m main column (80/100 mesh 3.2 mm OD $\times$ 2.2 mm I.D.), plumbed in the configuration described below, then separate the dissolved gas sample into O_2 and N_2 peaks. Typical oven temperatures and carrier gas flow rates are 55 °C and 25 ml min^{-1} respectively. Conditions were adjusted slightly for each batch of samples. The O_2 and N_2 peaks were quantified with a Perkin Elmer 8310 gas chromatograph equipped with a thermal conductivity detector.

The 10 port valve injects the seawater sample and then rotates to clear the stripping chamber after the apparatus has degassed the sample. Carrier gas flows into the 10 port valve through ports 5 and 10 (Fig. 2). In the 'load' position, the gas entering port 5 exits port 7 flows through the gas stripper and out port 8 to waste. The gas flowing to the detector is provided by the gas entering port 10. After loading the sample loop, the valve is rotated to the 'inject' position (Fig. 2 inset). The inject position reverses the direction of gas flow in the gas stripper. Carrier gas entering port 5 travels through the sample loop pushing the sample out port 6 into the gas stripper. The carrier gas bubbles through the gas stripper, passes through a 32 cm Drierite column, enters and exits port 7 and 9 respectively before entering the first MS-5A column. The digital timer holds the 10 port valve in this configuration until all the N_2 in the sample has reached the precolumn (empirically determined to be 1.2 min), the valve then returns to the 'load' position. The flow through the gas stripper is thus returned to the original direction. Carrier gas traveling from port 7 to waste pushes the degassed liquid sample back in port 6 and out port 8 where it collects in a liquid trap.

The 4 port valve maintains constant gas flow to the detector during the inject stage. When a liquid sample is injected into the gas stripper, the liquid travels much slower through the frit than carrier gas. To prevent this change in flow rate from affecting the detector, the 4 port valve temporarily changes the source, but not the flow rate, of gas to the detector. When a sample is injected the 4 port valve is manually rotated from the 'On line' position (Fig. 2) to the 'Vent' position (Fig. 2 inset). The ball valve connected to this valve is simultaneously opened. In the 'Vent' position, carrier gas from the precolumn escapes to waste; however, the presence of a precolumn sufficiently retards

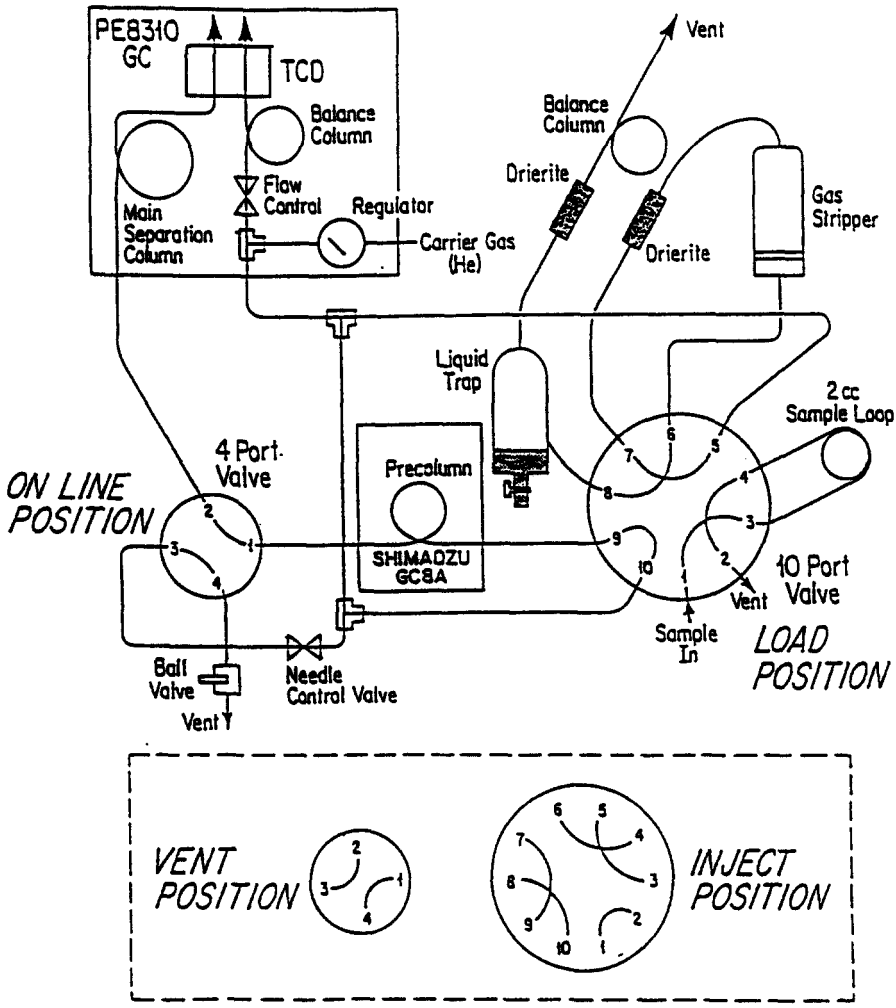

Fig. 2. Schematic of gas stripper design. The inset represents the alternative positions of the valves. When a sample is injected the valves are rotated to the positions shown in the inset.

the passage of O_2 and N_2 to prevent sample loss in the 45 sec of venting. The needle control valve controls carrier flow out of port 2 to keep gas flow to the detector constant as this venting occurs.

Calibration

Dissolved gas samples were calibrated with liquid and gas standards. This combination allowed calculation of a standard curve and assessment of the efficiency of the stripping apparatus. A high correlation coefficient between

liquid and gas standards would indicate that the stripper extracts dissolved gases completely. Liquid samples were made by equilibrating seawater with air at a fixed temperature. The concentration of O_2 and N_2 in the standards was then determined by using the equations of Weis (1970). A gas standard of 1% air in helium was prepared by Liquid Carbonic (Chicago, IL).

Ar was present in both standards and samples. This gas coelutes with O_2 in above ambient temperature molecular sieve columns, and thus was not separated from the O_2 peak. Because Ar is an inert gas, changes between samples in the Ar/O_2 peak were assumed to reflect oxygen metabolism.

Nutrients

Water samples were collected just above the sediments at the same site and time of each core sampling to obtain data so NO_3^- concentrations in overlying water at the start of each lab incubation could be matched to those found in the field. Water samples were collected with acid-washed plastic syringes, filtered through a 0.45 μM membrane filter, acidified with 10^{-3} volumes 5 N HCL and stored at 4 °C before nutrient analysis. NO_3^- and NO_2^- concentrations were determined colorimetrically with a Lachat autoanalyzer. The potential contribution of NO_3^- removal to N_2 flux was estimated by multiplying sediment nitrate removal rate by the ratio of 0.59 moles N_2 produced per mole of NO_3^- consumed (Devol & Christensen 1993).

Anaerobic controls

To test the hypothesis that coupled nitrification/denitrification produces the measured N_2 fluxes, flux measurements of cores in anaerobic conditions were conducted on four dates. O_2 in the overlying water was lowered to the detection limit of a YSI O_2 probe by bubbling the overlying sea table water with a mixture of 22.1% helium in N_2 (Liquid Carbonic, Chicago, IL) overnight. To remove any trace O_2, 0.03 g sodium bisulfite was added per liter of overlying water. After these treatments the cores were capped and headspace N_2 concentration monitored as above.

Prediction of temperature-driven flux

The possible sediment N_2 flux created by temperature changes in sediments was calculated by modelling the seasonal high and low sediment temperatures and calculating the N_2 pools at these temperatures, and by assuming that the porewaters came to equilibrium with the atmosphere. The losses and gains of N_2 across the sediment water interface that would accompany these shifts in sediment concentrations estimates the potential flux caused by temperature changes.

The seasonal temperature change in sediments was predicted by calculating the amplitude of variations in sediments as a function of the amplitude of bottom water temperature changes. Bottom water temperatures were measured with a YSI O_2 meter. The effective depth (D_e) and amplitude of temperature change was estimated by calculating the damping depth (D_d) from the one dimensional heat flow equation

$$D_d = (Pk/\pi)^{1/2} \quad (1)$$

where P is the period of the temperature wave (one year), k is the thermal diffusivity of sediments in $m^2\ sec^{-1}$), and $D_e = 2^{1/2}D_d$ (Monteith 1973). D_d is the depth at which the amplitude of temperature is e^{-1} of the surface amplitude. Literature values for k in muddy sediments range from 0.4 to $0.8 \times 10^{-6}\ m^2\ sec^{-1}$ (Harrison & Phizacklea 1987; Piccolo et al. 1993). Higher values represent either intertidal exposure or faunal activity (Harrison & Phizacklea 1987). Because the sampling sites in Waquoit Bay were subtidal and did not have large sediment faunal populations, k values of 0.47×10^{-6} $m^2\ sec^{-1}$ for muddy sediments were used (Harrison & Phizacklea 1987).

The amplitude of temperature changes at a sediment depth Z were calculated as a function of the amplitude of the surface change (Ao) by

$$A_1 = A_O e^{(-Z/D_d)} \quad (2)$$

where A_1 equals the amplitude at depth Z (Monteith 1973). This model was checked by comparing the predicted summer sediment temperature profile with direct measurements in the Childs River. On August 12, 1994 sediment temperature was measured *in situ* with a digital thermistor mounted on a dowel. Models of sediment temperature change were calculated from both literature values of k and by solving equations (1) and (2) for k given measured *in situ* sediment temperatures.

From these models the seasonal temperature maximum and minimum values were calculated over the respective effective depths by adding or subtracting A_1 from the mean bottom water temperature (13 °C). For these maximum and minimum temperatures, the equilibrium N_2 concentrations were calculated based on the solubility equations of Weis (1970). To estimate the N_2 flux that would accompany this concentration shift, the difference between the maximum and minimum concentrations was integrated over D_e. The sum of this difference was then divided by half the temperature cycle period and adjusted for the porosity of the sediments to estimate the average flux that would result from these changes in the sediment N_2 pools. Porosity was determined by weighing known volumes of wet sediment sections and by calculating wet/dry weight ratios.

Table 1. Denitrification for a variety of marine and coastal systems. Direct method refers to measurements of changes in N_2 from ambient. He flushing refers to method in which the background N_2 concentration is lowered. Fluxes are μmol N_2 m^{-2} h^{-1}. CV represents the average measurement error observed in flux estimates. Measurement error is defined as the percentage the standard error is of the rate estimation. Hyphen indicates data are not available.

Site	Method	Range	Mean	CV (%)	Reference
Shelf	Direct	18–97	55	–	Devol & Christensen (1993)
"	"	16–110	67	–	Devol (1991)
Estuary	He flushing	4–71	27	–	Yoon & Benner (1992)
"	"	24–97	62	4	Nowicki (1994)
"	Stoichiometry	–	67	–	Smith et al. (1991)
"	Acetylene	–	50	5	Seitzinger et al. (1993)
"	Isotope dilution	14–26	–	44	Nielsen (1992)
"	Direct	−9–712	125	35	Present paper
Fjord	Acetylene, N^{15}	102–727	326	–	Binnerup et al. (1992)

Results

Evaluation of method

Gas and liquid standards typically were highly correlated in standard curves; 12 of 19 standard curves had $r^2 > 0.999$. The remaining standard curves had coefficients greater than 0.995. High correlation coefficients and the smooth tail in the N_2 peak (Fig. 3) indicated that the apparatus effectively stripped dissolved N_2 from seawater. The dissolved gases stripped from the sample were completely separated into O_2 and N_2 peaks in under 10 min (Fig. 3). The complete removal of N_2 from a sample, and separation of this gas into a discrete peak, allowed precise measurements of N_2 in seawater. Standard deviation of duplicate measurements averaged less than $\pm 0.3\%$ of the mean.

The analytical precision of the gas stripping technique allowed detection of changes in N_2 concentration in water overlying sediments (Fig. 4). From these changes N_2 fluxes were estimated by a Model I regression of N_2 concentration versus time. Measurement error was calculated as the coefficient of variation (CV) of the N_2 flux rate by dividing the standard error of the time course slope estimator by the slope estimation. Of the 84 core measurements taken, no change or negative fluxes were detected 6 times. In 78 cores, in which N_2 release was measured, CV of N_2 flux averaged 35% of the mean (Fig. 5 top) which is within the range of average errors of other methods published in recent papers (Table 1).

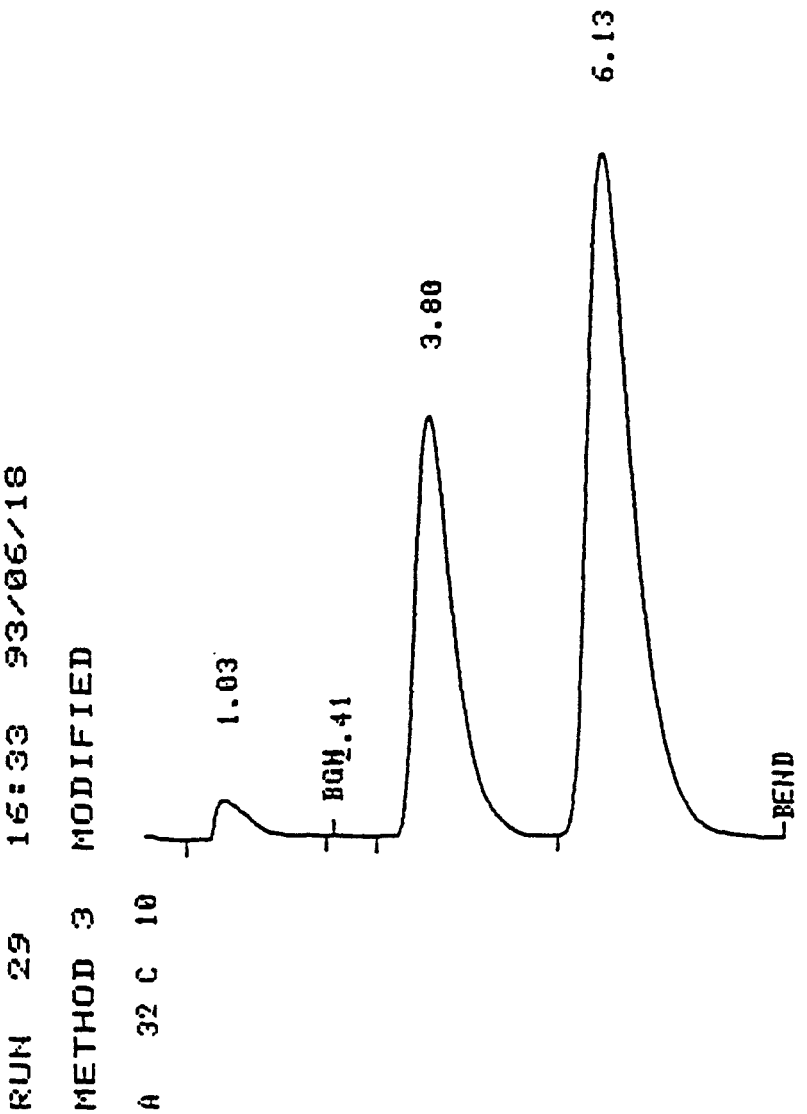

Fig. 3. Chromatogram from a typical gas analysis. The O_2 and N_2 peaks came out at 3.80 and 6.13 minutes respectively. The small peak at 1.03 min comes from the 4 port valve switching from 'On Line' to 'Vent' in the first minute of a sample run.

The highest measurement errors occurred when N_2 concentration changed little over the course of an incubation (Fig. 5 top). Most (92%, Fig. 5 top) N_2 concentrations changed less in the course of flux measurements than the average 5% change in shelf sediment incubations (Devol 1991). All of

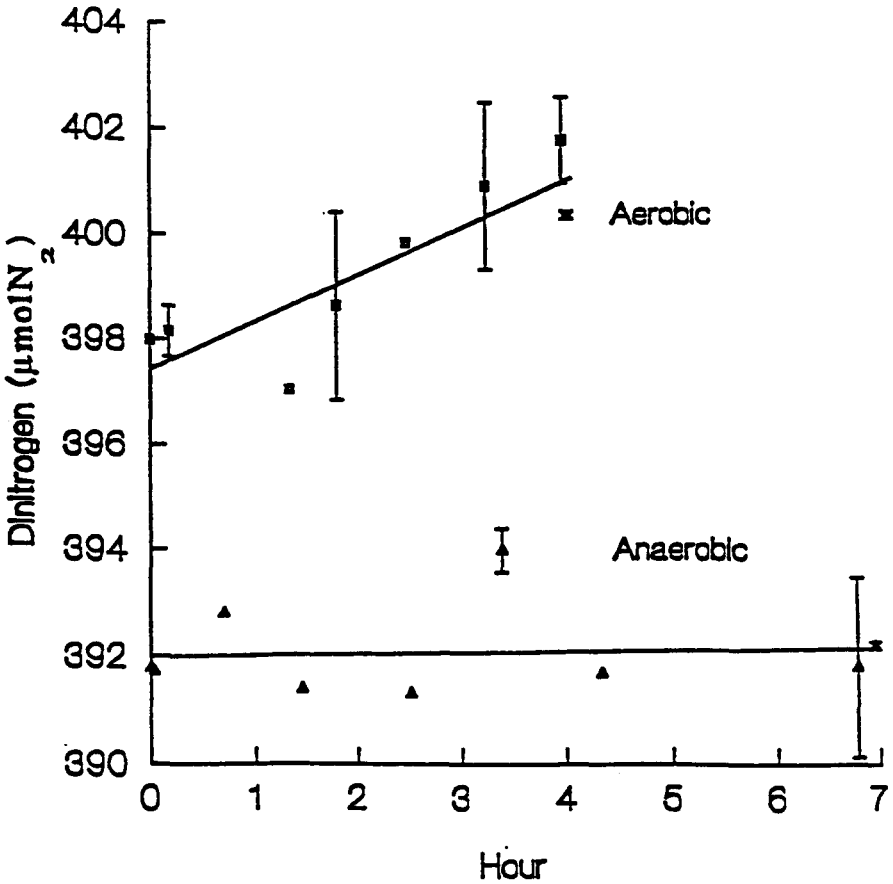

Fig. 4. N_2 change with time in the headspace of a core. Lines represent Model I regressions between mean concentration for each timepoint. Error bars represent the standard deviation of replicate analysis of samples. Graph shows cores incubated under aerobic and anaerobic conditions in August 1993. The lower initial concentration in the anaerobic incubation results from the ambient water warming.

the CVs greater than 100% occurred when the N_2 concentration changed less than 0.6%. These high measurement errors may reflect the analytical precision (0.3%) of the gas stripping apparatus.

Low percent changes occurred mostly when the ratio of N_2 to O_2 flux was low (Fig. 5 bottom). The percentage N_2 change covaried significantly (r^2 = 0.697) with the ratio of N_2 and O_2 fluxes. On several occasions low changes were measured despite relatively high N_2/O_2 flux ratios. These points resulted from incubations where available headspace O_2 was not exhausted.

Data from previous work and this study indicates that sediments from estuarine sediments, and Waquoit Bay in particular, have significantly lower

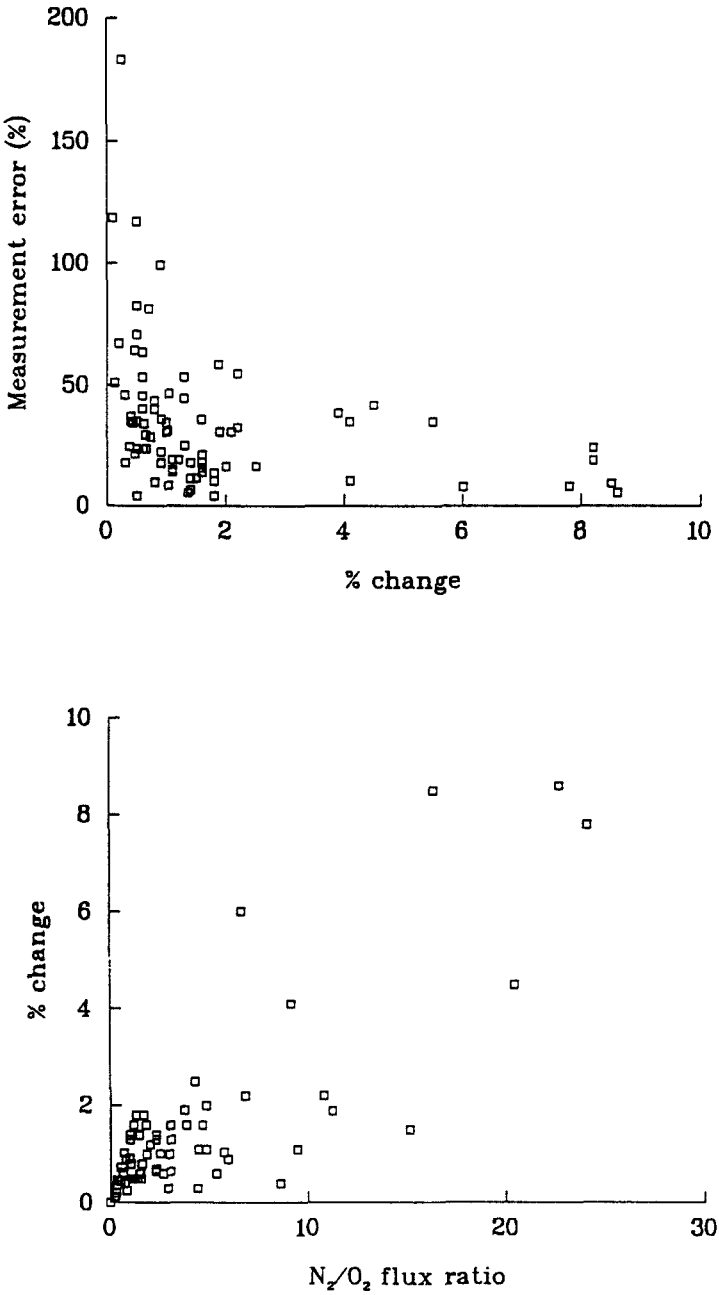

Fig. 5. Top. Percentage N_2 change versus measurement error. Percentage N_2 change is the total N_2 increase over the course of an incubation divided by the initial concentration. Measurement error was calculated by dividing the standard error in the N_2 flux rate by the flux rate. Bottom. N_2/O_2 flux ratio versus percentage N_2 change. N_2/O_2 ratio represents the ratio of N_2 production to O_2 consumption.

Table 2. Fluxes of N_2 and O_2 for two cores collected from Massachusetts Bay. Cores were collected 9 miles east of Boston Harbor (A. Giblin pers. comm.). Predicted N_2 flux was calculated by the difference between NH_4^+ mineralization and regeneration as per Banta et al. (1995) except mineralization was estimated by measuring dissolved inorganic carbon flux.

Measured N_2 (μmol N_2 m^{-2} h^{-1})	Predicted N_2	O_2 uptake (mmol O_2 m^{-2} h^{-1})	N_2/O_2 flux ratio (%)
65	62	0.74	8.8
44	36	0.58	8.6

N_2/O_2 than eastern Pacific continental shelf sediments ($P < 0.05$, Fig. 6). Unpublished preliminary evidence indicates that shelf sediments from Massachusetts Bay also have relatively high N_2/O_2 flux ratios (Table 2). The Waquoit Bay N_2/O_2 flux ratios were significantly lower than previous estuarine ratios ($P < 0.05$, Fig. 6).

Measurements of N_2 flux

N_2 flux ranged up to 712 μmol N_2 m^{-2} h^{-1} (Fig. 7 top). Only one other study (Binnerup et al. 1992) has reported rates as high as the maximum rate reported here (Table 1). The maximum rate measured represents a rare observation. Denitrification rates appear lognormally distributed; most of the observations have fluxes less than 100 μmol N_2 m^{-2} h^{-1} (Fig. 7 top).

N_2 flux in Waquoit Bay sediments appears dependent on O_2 not NO_3^- availability. N_2 flux increased 7-fold in the presence of O_2 (Table 3). In both aerobic and anaerobic incubations NO_3^- uptake was small relative to N_2 flux. In most of the aerobic incubations NO_3^- uptake from overlying water could have provided less than 5% of the observed N_2 flux (Fig. 7 bottom). In anaerobic incubations uptake of NO_3^- was an order of magnitude lower than N_2 flux. Indeed, because NO_3^- ammonification can account for a significant proportion of sediment NO_3^- uptake (Jørgensen 1989), measurements of NO_3^- uptake probably overestimate the contribution of sediment uptake to sediment N_2 release.

Temperature-driven N_2 flux

Temperature-driven changes in sediment N_2 pools were large enough to produce N_2 fluxes similar to those of denitrification. Using literature values of k, the thermal diffusivity equation (Eq. 1) predicted temperature varies

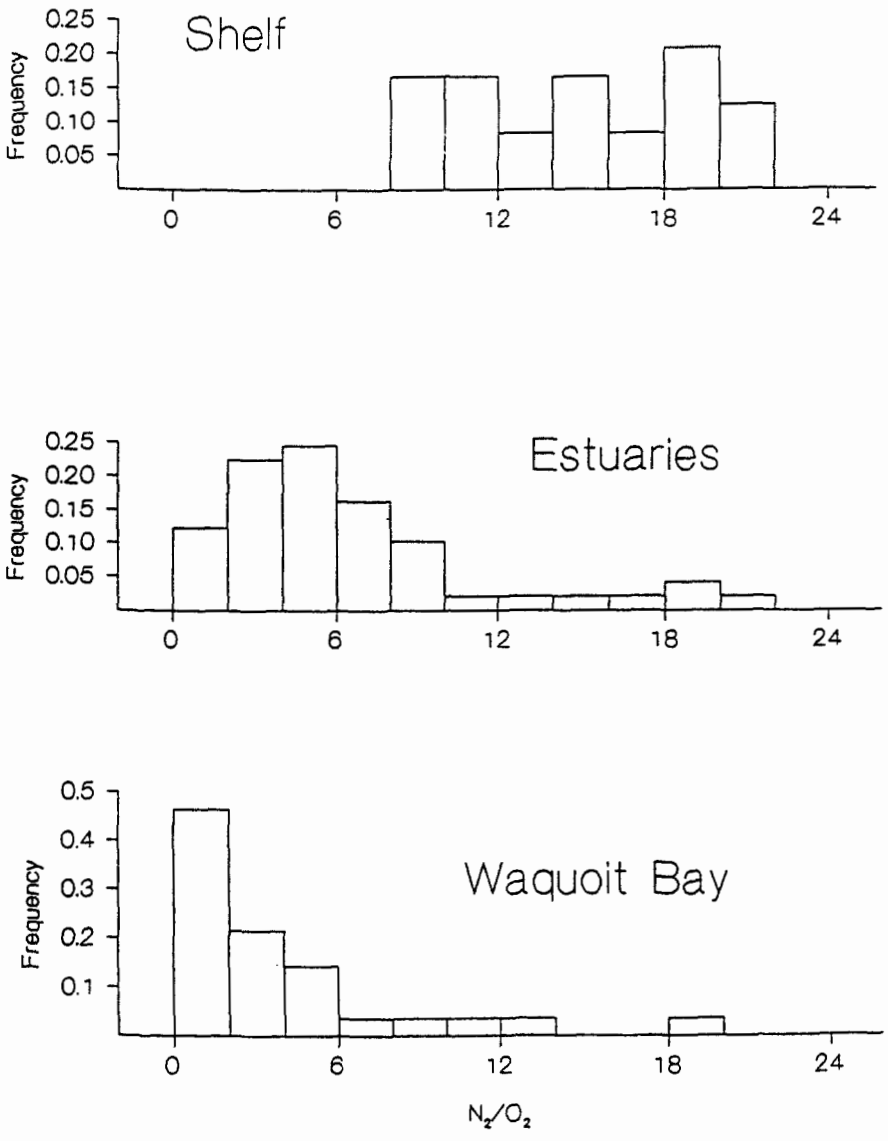

Fig. 6. Ratio of N_2 flux to O_2 consumption for a variety of marine sites. Shelf data from Devol (1991) and Devol & Christensen (1993). Estuarine data from Yoon & Benner (1992), Seitzinger et al. (1984), and Smith et al. (1991). Waquoit data from fluxes measured in this study.

seasonally to a depth of 3.1 m; *in situ* measurements indicate field temperatures change less (Fig. 8). Assuming a bottom water amplitude of 13 °C, and solving equations (1) and (2) for k, k averaged 0.2×10^{-6} m^2 sec^{-1} for the sediments of Waquoit Bay. From this lower thermal diffusivity sediment

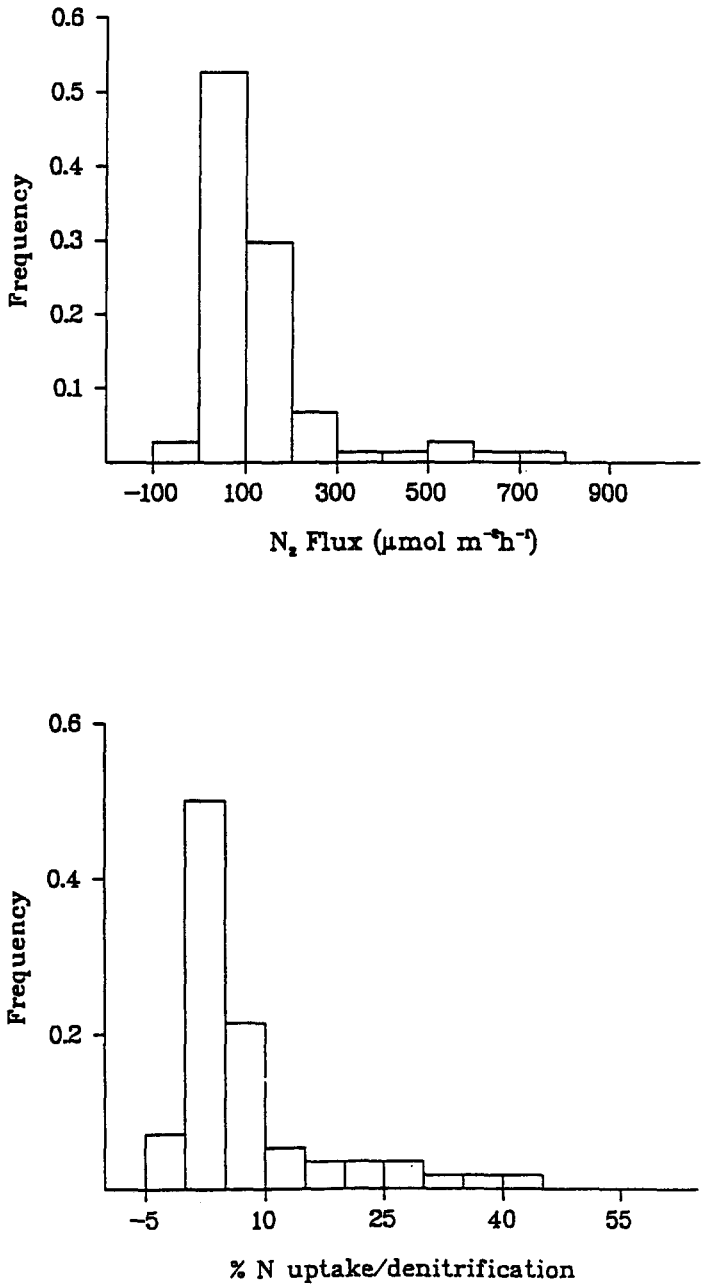

Fig. 7. Top. Frequency of denitrification rates. Measurements were made throughout the year. Most measurements were made in the summer. The seasonal pattern of denitrification is discussed elsewhere (LaMontagne 1996). Bottom. Percentage of N_2 flux due to direct denitrification. Direct denitrification was calculated as the amount of N_2 flux predicted based on the rate of nitrate removal from the overlying water.

Table 3. Comparison of N_2 flux ($\mu \pm$ SD) under aerobic and anaerobic conditions. Error represents the standard deviation (SD) of replicate aerobic and anaerobic cores. Two cores were used for each treatment. N_2 expected was predicted based on the average NO_3^- uptake rate in anaerobic cores according to the ratio of 0.59 moles N_2/mole NO_3^- as per Devol & Christensen (1993).

Date	N_2 flux (μmol N_2 m^{-2} h^{-1})		
	Measured		Expected
	Aerobic	Anaerobic	
14-May-93	89	15	0.6
17-Aug-93	149 ± 56	8 ± 5	0.3
28-Jun-94	100 ± 2	25 ± 31	4.1
13-Jul-94	223 ± 125	32 ± 15	1.3

temperatures were predicted to vary significantly to 1.9 m. For porewater to reach equilibrium with the atmosphere at the maximum and minimum temperatures over this depth, sediments would have to gain and lose 192 mmol N_2 m^{-2}. These changes represent a potential average flux of 43 μmol N_2 m^{-2} h^{-1}. This rate is in the range of fluxes measured in cores (−9 to 712 μmol N_2 m^{-2} h^{-1}).

Discussion

The ability to detect changes above ambient N_2 concentration of water overlying estuarine sediments allows quantification of denitrification in these systems. The error in estimates of rates with this method will depend, in part, on how much change in N_2 occurs during incubations. Low percent change, and subsequent high measurement error, reflects the low N_2/O_2 flux ratio in estuarine sediments. The difference in relative importance of denitrification in total sediment metabolism between shelf and estuaries could reflect high NO_3^- uptake rates in shelf sites. While NO_3^- could support only 5% of N_2 flux in most of the estuarine measurements reported here (Fig. 7 bottom), NO_3^- uptake averaged 47% of the N_2 flux in shelf sites (Devol 1991). Depth could explain the difference in sediment NO_3^- uptake between shelf and estuarine sites. In estuaries bottom waters are often within the photic zone and thus primary producers can reduce nitrate concentrations. Shelf bottom waters are beneath the photic zone, and thus nitrate is available for benthic uptake.

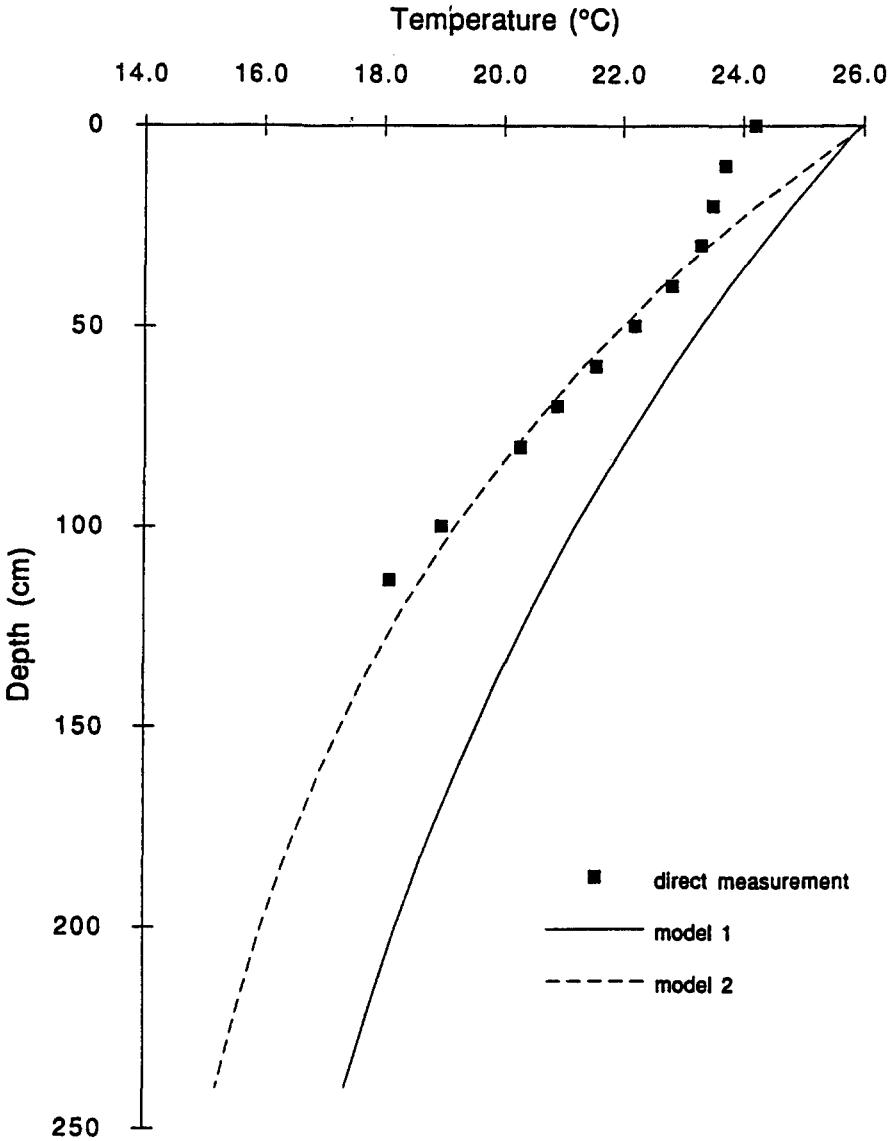

Fig. 8. Sediment temperature profiles. Direct measurements of sediment temperature were compared to profiles predicted from literature values of k (model 1) and k calculated from *in situ* measurements (model 2).

To ascertain if the rates of denitrification reported here are reasonable, we compared our data with two other independent data sets. First, K. Gribble and K. Foreman (unpubl. data) measured sediment ammonium production (mineralization) and sediment ammonium fluxes (regeneration) in the Childs

River. The difference between these 2 measurements, 150 μmol N_2 m^{-2} h^{-1}, was assumed to have been denitrified. This estimate agrees with the average fluxes (140 and 170 μmol N_2 m^{-2} h^{-1}) we measured during comparable times of two years. Second, denitrification estimated in Massachusetts Bay cores, obtained by comparing regeneration and mineralization rates, agree with direct measurements of N_2 flux (Table 2).

Microbial processes such as N_2 fixation and N_2O release could bias estimates. N_2 fixed would be lost from the N_2 pool in the overlying water and result in an underestimation of denitrification. The rate of N_2 fixation, though, appears small compared to denitrification rates. N_2 fixation rates in temperate marine macrophyte-free sediments range from 1.6–6.5 μmol N_2 m^{-2} h^{-1} (Howarth et al. 1988), an order of magnitude lower than denitrification rates reported in this paper.

Microbes release N_2O in both nitrification and denitrification. N_2O release has been reported for eutrophic estuarine systems (Seitzinger et al. 1984; Seitzinger 1988), and, if unaccounted for, could lead to underestimation of denitrification. Waquoit Bay sediments consume N_2O (LaMontagne 1996) at rates several orders of magnitude lower than the denitrification rates reported for Waquoit Bay. Because the rate of N_2O uptake is relatively low, the N_2 produced should not bias estimates of denitrification.

The control fluxes indicate that either the sediment temperature model overestimates temperature driven fluxes or anaerobic controls underestimate non-denitrification N_2 fluxes. The model fit to *in situ* temperatures overestimates temperature changes near the surface and below 1 m (Fig. 8). Furthermore, disequilibrium created by temperature changes may not result in diffusive fluxes. Sediment N_2 pools could also come to equilibrium, without resulting in a diffusive flux across the sediment/water interface, through advective transport by methane ebullition (Kipphut & Martens 1982). Given that N_2 release from anaerobic controls averaged only 14% of the aerobic fluxes (Table 3), the error introduced by non-denitrification fluxes appears less important than the measurement error in rates. However, as discussed previously, anaerobic incubations could bias control rates by inhibiting infaunal activity (Nowicki 1994). The sediments of Waquoit Bay did not appear to have a large infaunal community, so that this effect was probably small for these sediments. Alternative controls should be considered in systems with abundant benthic fauna.

Measurements of N_2 flux can be used to estimate denitrification, but fluxes are hard to detect when sediment oxygen demand is relatively high. Anaerobic controls indicate N_2 flux in Waquoit Bay results from coupled nitrification/denitrification. Uptake of NO_3^- from the overlying water was relatively small. The N_2 flux observed agrees with independent estimates of

denitrification for this system. Temperature changes could create an abiotic N_2 flux in the range of denitrification rates observed in this study; however, anaerobic controls indicate that the error introduced by temperature-induced fluxes is likely to be small.

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