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Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas)

Soonmo An*, Wayne S. Gardner

The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, Texas 78373, USA

ABSTRACT: Rates of nitrate (NO_3^-) reduction to nitrogen gas (N_2) and ammonium (NH_4^+) were measured in August and December 1999 on intact cores (Laguna Madre and Baffin Bay, Texas) using flowing seawater enriched with $^{15}NO_3^-$. The combination of membrane inlet mass spectrometry (MIMS) and high performance liquid chromatography (HPLC) allowed accurate and simple estimation of these 2 dissimilatory pathways of NO_3^- reduction. NO_3^- enrichment (~100 µM $^{15}NO_3^-$) did not stimulate denitrification (mean ± SE = 55 ± 16 and 69 ± 15 [Aug 99], −11 ± 16 and 11 ± 18 [Dec 99] µmol N m^{-2} h^{-1} before and after $^{15}NO_3^-$ addition, respectively; n = 8). However, $^{15}NH_4^+$ production rates increased after the $^{15}NO_3^-$ addition (69 ± 14 [Aug 99], 50 ± 9 [Dec 99] µmol N m^{-2} h^{-1}), comprised about 1/3 of total NH_4^+ flux, and were comparable to denitrification rates. A larger portion of added $^{15}NO_3^-$ was converted to $^{15}NH_4^+$ (15 to 75%) than to N_2 ($^{29+30}N_2$; 5 to 29%) on both sampling dates. High dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and low denitrification suggest that sulfide may influence the processes. High sulfide concentrations inhibit nitrification and denitrification but may enhance DNRA by providing an electron donor. Inhibited denitrification and enhanced DNRA may preserve available nitrogen in Laguna Madre/Baffin Bay, which has limited water exchange with other bodies of water.

KEY WORDS: Dissimilatory nitrate reduction to ammonium · DNRA · Denitrification · Brown tide · Nitrogen conservation · Laguna Madre/Baffin Bay

INTRODUCTION

The relative partitioning between the 2 major pathways of nitrate (NO_3^-) reduction can help determine the degree of available nitrogen (N) conservation in shallow estuaries (Tiedje et al. 1982, Sørensen 1987, Jørgensen 1989, Patrick et al. 1996, Tobias et al. 2001). When NO_3^- is consumed by denitrification, 'total available' N is decreased since the final product (N_2 gas) is less available for biological production than ammonium (NH_4^+) or NO_3^- (Howarth et al. 1988). However, if NO_3^- is used during dissimilatory NO_3^- reduction to NH_4^+ (DNRA), N will be conserved in a form that is available to organisms (Koike & Hattori 1978, Jørgensen 1989, Patrick et al. 1996). Although the occurrence of DNRA has been demonstrated in marine sediments (e.g. Koike & Hattori 1978, Sørensen 1978, Tobias et al. 2001), the ecological significance of the process is not understood (Sørensen 1987, Cornwell et al. 1999). DNRA rates may be as high as denitrification in shallow estuaries and tidal flat sediments (Koike & Hattori 1978, Kasper 1983, Jørgensen 1989, Rysgaard et al. 1996, Bonin et al. 1998, Tobias et al. 2001).

Laguna Madre/Baffin Bay is the largest estuary in Texas but receives no major river discharges. Despite the lack of known nutrient inputs, this region is nutrient replete and has suffered from a long lasting bloom of Texas Brown Tide. This monospecific algal bloom of

*Present address: Kyungnam University, Department of Environmental Engineering, Masan, Kyungnam, South Korea 631-701. E-mail: sman@kyungnam.ac.kr

Aureomonas lagunensis started in January 1989 and continued for about a decade (Buskey et al. 1998), causing a decline in seagrasses and other benthic fauna (Montagna & Kalke 1995). The persistence of an algal bloom in this area is unusual and the cause of the bloom is not fully understood. *A. lagunensis* has the unique characteristic of being able to use NH_4^+ or NO_2^- but not NO_3^- as a nitrogen source (DeYoe & Suttle 1994). We hypothesize that *A. lagunensis* can outcompete other autotrophs in this system because of its reliance on reduced N forms. The DNRA mechanism, by supplying NH_4^+, could help explain the success of Texas Brown Tide.

One reason that DNRA is not understood fully in coastal systems is that methods have been inconvenient. Using $^{15}NO_3^-$ as a tracer and measuring $^{15}NH_4^+$ production rates (for DNRA) have required extensive equipment and effort (Sørensen 1987, Jørgensen 1989, Binnerup et al. 1992, Bonin et al. 1998). Although acetylene inhibition for denitrification measurement is simple and sensitive, problems are associated with the technique including its inhibition of coupled nitrification-denitrification (Knowles 1990). Membrane inlet mass spectrometry (MIMS) allows accurate and simple measurement of N_2:Ar changes due to denitrification (Kana et al. 1994). In this study, we expanded the capability of this technique by measuring different isotopic forms of N_2 gas ($^{29}N_2 = {}^{14}N + {}^{15}N$ and $^{30}N_2 = {}^{15}N + {}^{15}N$) relative to Ar (An et al. 2001). The modified setup allowed mechanistic experiments involving $^{15}NO_3^-$ addition to be conducted. By adding the tracer, the denitrification measurement estimated from the N_2:Ar ratio can be verified with an isotope pairing technique (Nielson 1992). In addition, the simultaneous measurement of N fixation and denitrification is feasible (An et al. 2001). Using the modified MIMS and high performance liquid chromatography (HPLC) to measure $^{15}NH_4^+$ in water samples (Gardner et al. 1995), we could measure the 2 major NO_3^- reduction processes (denitrification and potential DNRA) in Laguna Madre/Baffin Bay with a flow-through sediment incubation chamber (Kana et al. 1994, Lavrentyev et al. 2000). Here, we report the results of sediment incubation experiments conducted in August and December 1999 and discuss the potential interaction between the fate of NO_3^- and the presence of sulfide, which occurs in the study region.

MATERIALS AND METHODS

Laguna Madre/Baffin Bay is a shallow, semi-enclosed estuary located in southern Texas. Water exchange with the Gulf of Mexico is limited and it is a negative estuary where freshwater input is less than evaporation (residence time = 1 yr). The salinity is often more than 40 ppt and may reach up to 60 ppt (Buskey et al. 1998). Four stations in Laguna Madre and Baffin Bay were selected to measure water column characteristics (temperature, salinity and dissolved oxygen using a Hydrolab®) and conduct sediment core incubation experiments (Fig. 1). Stn B24 in Baffin Bay has a depth of 2.2 m and represents the deepest part of the bay. Stn B6 is shallow and the salinity of the overlying water was higher than B24. The sediment type at Stn B24 is fine clay, whereas Stn B6 has a high sand content (Table 1). The water depths at Laguna Madre stations were between 0.8 and 0.9 m and did not show spatial variability. Laguna Madre stations were populated with *Thalassia testudinum* (300 to 600 shoots m^{-2}; Lee & Dunton 1999). Sand content was highest at Stn L155 among study sites (Table 1). Stn L189 had higher sand content than Stn B24 but lower than Stn B6. During our sampling period, salinity was lower than observed by Buskey et al. (1998) and did not exceed 40 ppt (Table 1). Bottom water was oxygenated at most stations due to wind-driven mixing, but oxygen concentrations were low in Baffin Bay during the summer, perhaps because of ground water (low salinity, low oxygen and high NO_3^- water) intrusion rather than increased sediment oxygen demand (Dr. D. Brock, Texas Water Development Board, pers. comm.). Baffin Bay salinity was lower in bottom versus surface water at this time.

Undisturbed sediment cores (7.6 cm diameter, 30 cm length; 4 Stn^{-1}) with bottom water were collected using SCUBA gear. Within 3 h of collection, the cores were transported to the laboratory and a flow-through plunger with Teflon inlet and outlet tubes was installed

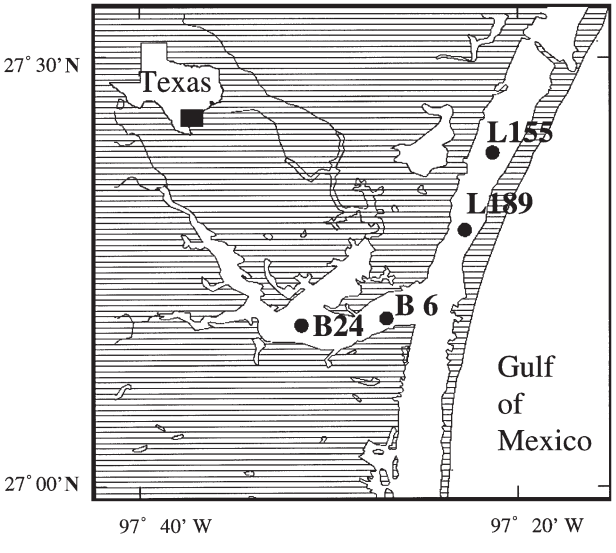

Fig. 1. Sampling stations in Baffin Bay (B6, B24) and Laguna Madre (L155, L189)

Table 1. Location and environmental variables in Laguna Madre (L155 and L189) and Baffin Bay (B6 and B24) in August and December 1999

	B6		B24		L155		L189	
	Aug	Dec	Aug	Dec	Aug	Dec	Aug	Dec
Location								
Longitude (W)	97.25.39.9		97.33.11.1		97.21.25.1		97.23.17.7	
Latitude (N)	27.16.46.7		27.15.47.6		27.24.34.2		27.20.53.1	
Water depth (m)	2.2		1.8		0.8		0.9	
Sand content (%; >64 µm)	33.9		5.2		72.4		12.1	
Salinity (ppt)								
Surface	35.23	30.04	31.6	25.67	38.07	31.94	37.18	30.52
Bottom	19.2	30.05	24.11	25.72	38.14	32.09	37.34	31.37
Temperature (°C)								
Surface	31.06	16.21	30.62	15.8	30.18	16	29.54	15.64
Bottom	30.75	16.19	30.36	15.75	30.22	15.59	29.56	15.79
Dissolved oxygen (mg l^{-1})								
Surface	5.77	7.66	5.5	6.95	5.35	6.58	4.66	6.76
Bottom	1.35	7.09	4.28	5.61	5.32	5.88	4.53	6.33
NO_3^- concentration (µM)								
Surface	0.72	0.12	0.65	0.13	0.58	0.58	0.57	0.56
Bottom	6.24	1.58	22.4	5.42	0.53	0.51	0.51	0.63

over each sediment core (Lavrentyev et al. 2000). The flow-through chamber setup consisted of an intake water vessel, Teflon flow tubes, a peristaltic pump, a temperature controlled incubation bath and a sample collection vessel. Sediment cores were placed in the incubation bath at *in situ* temperature, and bottom water collected from the site was passed over the core surface at a rate of 1.2 ml min^{-1}. Half of the cores were incubated under dim light (~30 µE m^{-2} s^{-1} = average light intensity in Baffin Bay; An & Gardner 2000) while the others were wrapped with aluminum foil. Water column depth over the sediment was ~5 cm to give a water volume of ~570 ml in each core. After the cores were incubated for 1 d to allow steady-state conditions to develop, triplicate samples of feed and outlet water were collected at intervals for dissolved gas analysis. Additional water samples were collected for analysis of dissolved inorganic nitrogen compounds (NH_4^+, NO_3^- and NO_2^-) using an automated Lachat FIA analyser.

After the 1st or 2nd day of sampling, feed water was enriched with $^{15}NO_3^-$ (99 at.% ^{15}N; $K^{15}NO_3$ from Aldrich USA) and the concentrations of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$ and $^{15}NH_4^+$ were measured in inflow and outflow waters. During denitrification, 3 different masses of N_2 gas can be produced ($^{28}N_2$ from $^{14}NO_3^-$, $^{30}N_2$ from $^{15}NO_3^-$, and $^{29}N_2$ from $^{14}NO_3^-$ and $^{15}NO_3^-$; Nielson 1992). Dissolved N_2, O_2 and Ar were measured by MIMS using methods modified from Kana et al. (1994) and described in An et al. (2001). The MIMS is equipped with quadruple mass spectrometer detection via a Channeltron/Faraday secondary electron multiplier (Balzers® Prisma QME 200). By using MIMS and Ar as an internal standard, errors associated with dissolved gas extraction and atmospheric contamination are reduced. The MIMS procedure is also fast, simple and precise (Kana et al. 1994). We modified the method of Kana et al. (1994) to measure dissolved $^{29}N_2$ and $^{30}N_2$ in addition to Ar, O_2 and $^{28}N_2$ (An et al. 2001). The $^{29}N_2$ and $^{30}N_2$ concentrations were obtained from the 'excess' atomic mass unit (amu) 29 and 30 signals, respectively, caused by the conversion of added $^{15}NO_3^-$. The ratio between $^{29}N_2$ and $^{28}N_2$ is 0.00732 (0.00366 × 2) in natural samples, considering that the natural abundance of ^{15}N is 0.366 % (Lide 1992). The relationship between $^{29}N_2$ and $^{28}N_2$ concentrations was obtained from standard water (30 ppt artificial seawater held at 21 and 30°C; Fig. 2a). This relationship was used to determine the excess amu 29 in a water sample. The feed water does not have excess amu 29 resulting from $^{29}N_2$ while outflow water has excess amu 29 indicating the presence of $^{29}N_2$ produced during denitrification (Fig. 2a). The excess amu 29 signal was converted to excess $^{29}N_2$ concentration by comparing results with those from standard water. The NO^+ ions (amu 30) formed from N_2 and O^+ inside the mass spectrometer caused a linear relationship between amu 30 and (amu 28 × amu 32)$^{0.5}$ (Jensen et al.

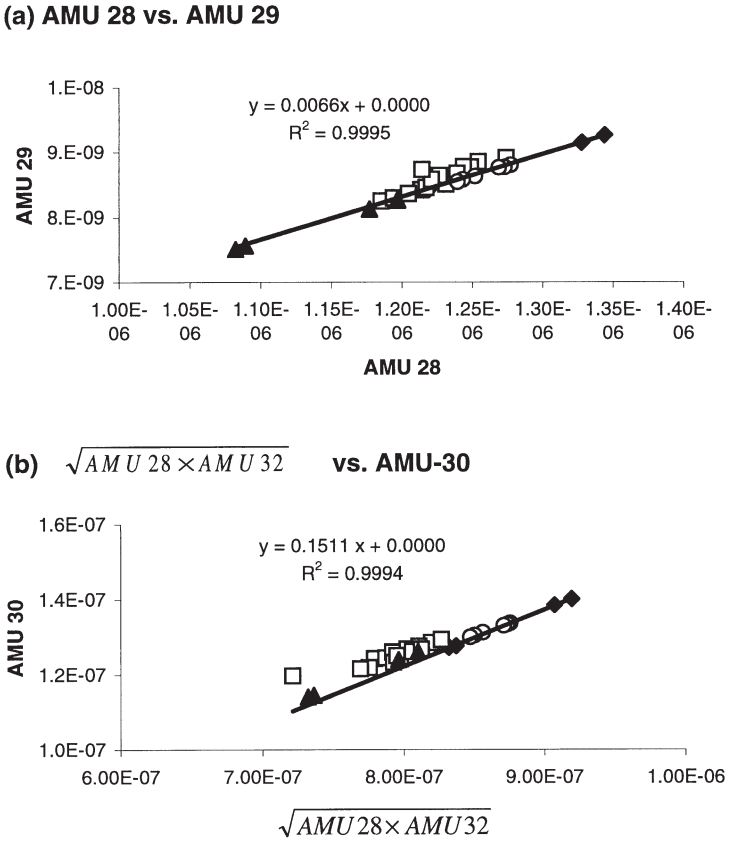

Fig. 2. Relationship between different atomic mass unit (amu) signals in liquid samples measured with a quadruple mass spectrometer. The solid symbol represents standard water (■: 30 ppt, 21°C; ▲: 30 ppt, 30°C) hollow symbols represents samples (□: outflow water; ○: inflow water). The line represents the regression among the standard water. (a) amu 28 versus amu 29 signal, (b) $\sqrt{\text{amu 28} \times \text{amu 32}}$ versus amu 30 signal. The signal represents partial pressure of the particular gas (mbar) in the mass spectrometer

1996). Jensen et al. (1996) suggested that a linear relationship occurs between amu 30 and (amu 28 × amu 32), but we found that (amu 28 × amu 32)$^{0.5}$ exhibited a better regression with amu 30 than (amu 28 × amu 32) (Fig. 2b, taken from An et al. 2001). If oxygen (O) and N atoms are combined randomly as N_2, NO and O_2, and the proportions O and N are p and q, respectively, then the proportions of the 3 products should show a binomial distribution ($N_2 = p^2$, $NO = 2pq$, $O_2 = q^2$), and account for the observed square root relationship of N_2O_2 with NO. This relationship was used to determine the excess amu 30 in the water samples. The excess amu 30 was converted to excess $^{30}N_2$ concentration by comparing values with those of standard water. The results of a sediment incubation experiment designed to calibrate the MIMS signal showed that the sensitivities for the 3 forms of N_2 gases are similar (An et al. 2001).

Concentration and at.% ^{15}N for NH_4^+ were determined in water samples by high performance liquid chromatography (HPLC; Gardner et al. 1995). This cation exchange method quantifies the at.% ^{15}N of NH_4^+ by measuring the shift in retention time of NH_4^+, relative to an internal standard, caused by the presence of $^{15}NH_4^+$ in the water sample. Concentration of total NH_4^+ is quantified by comparing the size of the sample NH_4^+ peak to that of the internal standard. This procedure is convenient to measure NH_4^+ concentration and isotope ratio in water. The sample filtrate is injected directly into the HPLC system without the need to isolate the NH_4^+ from the water and convert it to N_2 gas before analysis as is required for mass spectrometry. Sediment flux was calculated based on the concentration difference between feed water and outflow water, flow rate, and cross-sectional area (Lavrentyev et al. 2000).

RESULTS

The dim light condition did not cause significant light effects so data from the 2 treatments (dark and dim light incubation) were combined. Light effects were also not observed during an *in situ* sediment core incubation experiment using light-dark benthic chambers in the study area (An & Gardner 2000). However, light effects may have been underestimated in Laguna Madre where the light intensity was higher than in Baffin Bay. Sediment oxygen demand (SOD) was higher in August 1999 (964 ± 104 µmol m^{-2} h^{-1}) than December 1999 (546 ± 33 µmol m^{-2} h^{-1}; Fig. 3). Laguna Madre had a higher SOD (1069 ± 68 and 621 ± 13 µmol m^{-2} h^{-1} in August and December 1999, respectively) than Baffin Bay (858 ± 78 and 489 ± 15 µmol m^{-2} h^{-1} in August and December 1999, respectively). SOD tended to increase after the $^{15}NO_3^-$ addition, but the difference was not significant for most stations (Fig. 3; *t*-test $p > 0.05$). In August 1999, SOD at Stn B24 decreased from 1154 ± 109 µmol m^{-2} h^{-1} before the $^{15}NO_3^-$ addition to 432 ± 52 µmol m^{-2} h^{-1} after the $^{15}NO_3^-$ addition. In December 1999, fluxes were monitored for 3 d in Laguna Madre (Fig. 3d) and for 6 d in Baffin Bay (Fig. 3c). SOD increased significantly in Baffin Bay Stns B6 and B24 on Day 6 (4 d after the $^{15}NO_3^-$ addition) relative to Day 3 (1 d after the addition; Fig. 3c; *t*-test $p < 0.05$). In Laguna Madre, SOD was not significantly different on Day 2 (1 d after addition) from Day 3 (Fig. 3d; *t*-test $p > 0.05$).

Figs. 4 & 5 show the heavy N_2 gas ($^{29+30}N_2$) flux produced during $^{15}NO_3^-$-based denitrification and $^{15}NH_4^+$ flux produced from $^{15}NO_3^-$ via DNRA, respectively. As expected, neither process was measurable before the $^{15}NO_3^-$ addition. After the $^{15}NO_3^-$ addition, average

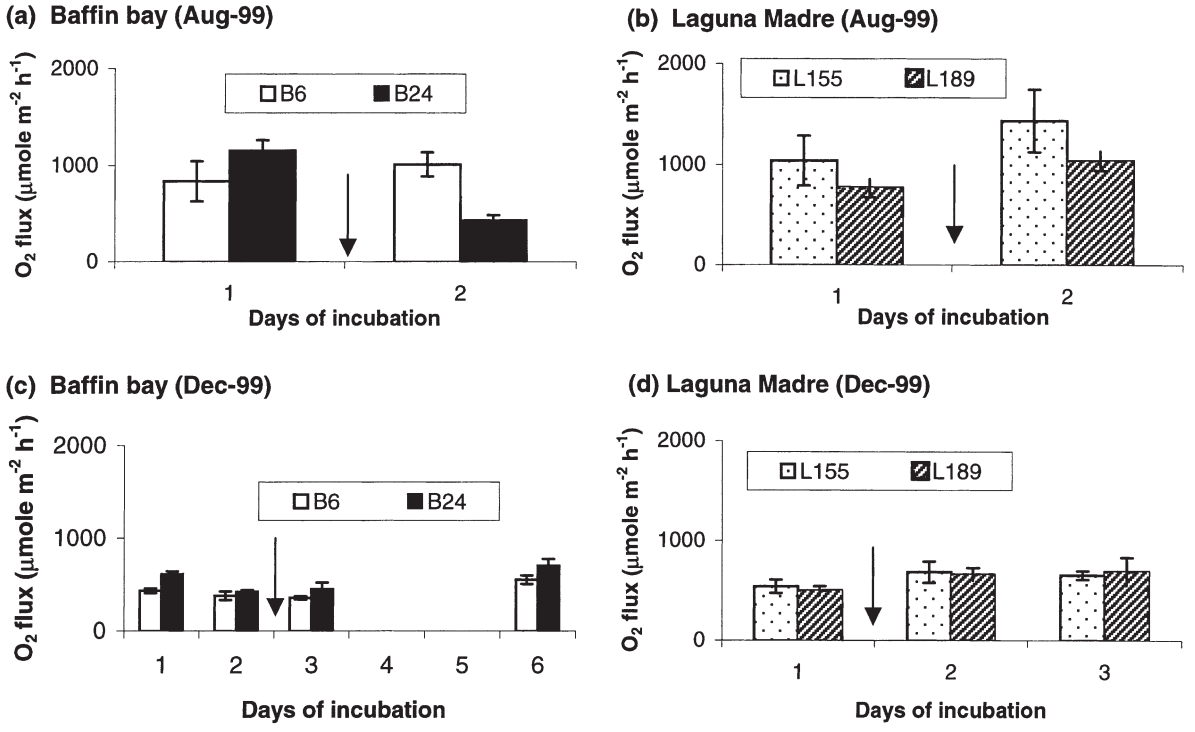

Fig. 3. Sediment oxygen demand (µmol m^{-2} h^{-1}) before and after $^{15}NO_3^-$ addition. Arrows show the time of addition. Bars represent average flux and lines represent ±1 SE among 4 sediment cores

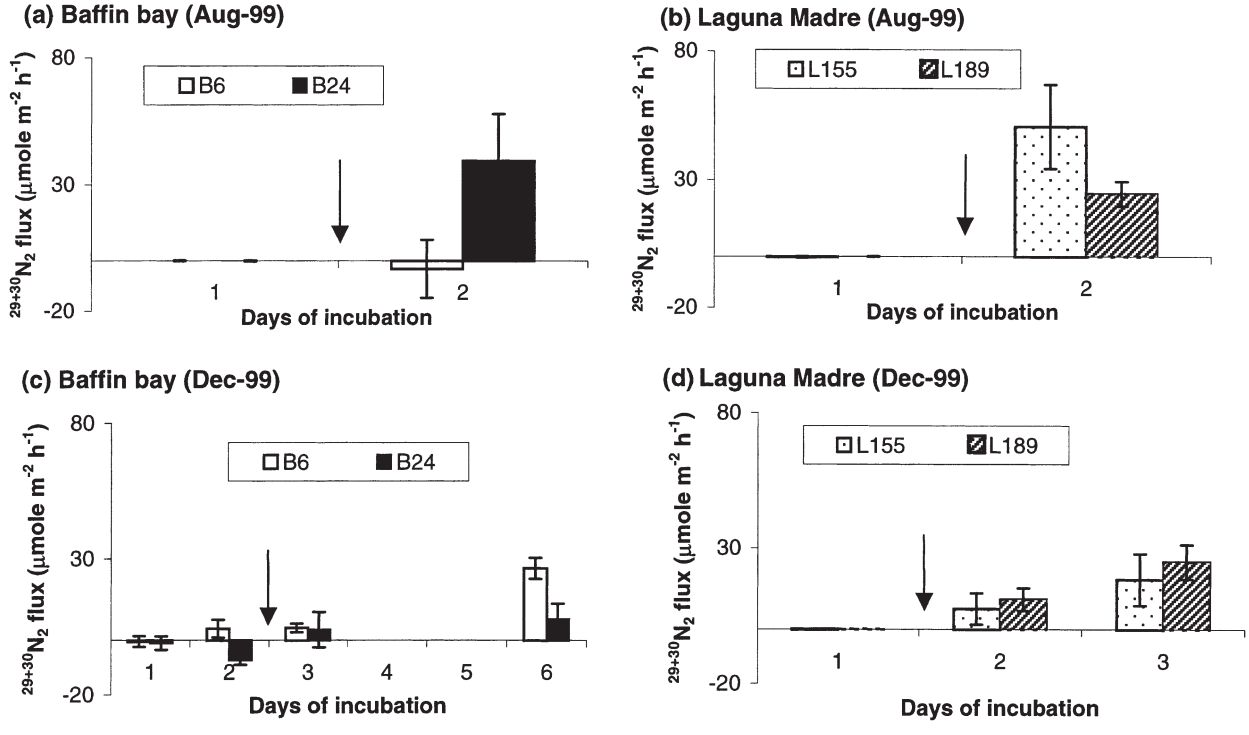

Fig. 4. Denitrification rate of $^{15}NO_3^-$ ($^{29}N_2$ plus $^{30}N_2$ production) before and after $^{15}NO_3^-$ addition. Arrows show the time of addition. Bars represent average and lines represent ±1 SE among 4 sediment cores

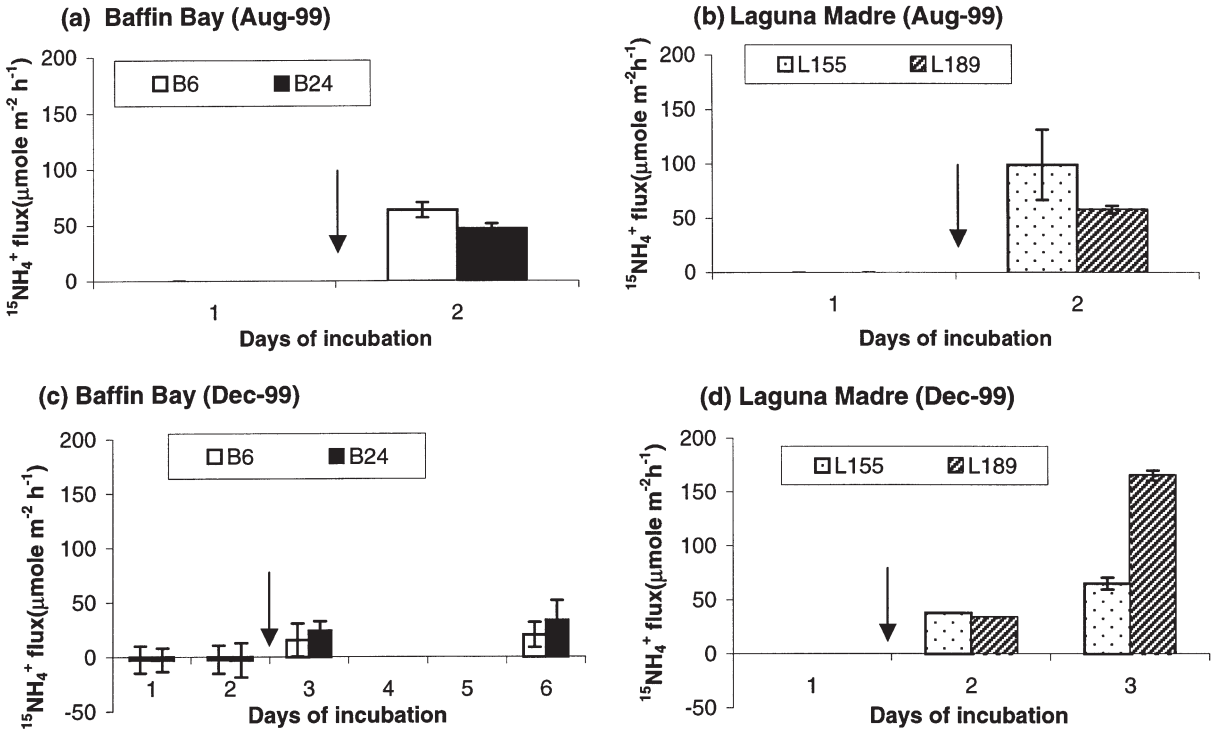

Fig. 5. Heavy ammonium ($^{15}NH_4^+$) flux before and after $^{15}NO_3^-$ addition. Arrows show the time of addition. Bars represent average and lines represent ±1 SE among 4 sediment cores

Table 2. Nitrogen gas ($^{28}N_2$ and $^{29+30}N_2$), NO_3^- and $^{15}NH_4^+$ flux (μmole -N equivalent m^{-2} h^{-1}) during sediment incubation experiments in August and December 1999. Average flux (standard error) among replicate cores (n = 16 to N 32) were presented before and after the $^{15}NO_3^-$ addition. Negative flux denotes a flux into the sediment. Percent of recovery for $^{29+30}N_2$ and $^{15}NH_4^+$ was calculated assuming that all the NO_3^- flux consisted of $^{15}NO_3^-$

	$^{28}N_2$ flux	NO_3^- flux	$^{29+30}N_2$		$^{15}NH_4^+$		% $^{15}NO_3^-$ recovery
			Flux	% of NO_3^- flux	Flux	% of NO_3^- flux	as N_2 + NH_4^+
August							
Baffin Bay							
Before	67.8 (15.8)	0.74 (1.3)	0 (0)		0 (0)		
After	47.5 (6.5)	–370 (103)	18.2 (21.3)	4.9	55.4 (8.3)	15.0	19.9
Laguna Madre							
Before	41.3 (14.3)	0.23	–0.12 (0.04)		0		
After	35.3 (20.3)	–128 (79)	37.6 (13)	29.4	78.6 (20)	61.4	90.8
December							
Baffin Bay							
Before	–2.3 (9.3)	1.2 (0.3)	–1.05 (2.7)		–2.5 (0.37)		
After	3.1(10.5)	–146 (4.3)	10.7 (5.4)	7.3	23.3 (3.8)	16.0	23.3
Laguna Madre							
Before	–19.9 (5.2)	0.13 (0.04)	0.34 (9.2)		–0.7 (0.9)		
After	–9.0 (8.8)	–101.7 (33)	15.5 (9.9)	15.2	76.1 (18)	74.8	90.1

fluxes were 10.7 to 37.6 and 23.3 –to 76.8 μmol m^{-2} h^{-1} for $^{29+30}N_2$ and $^{15}NH_4^+$, respectively (Table 2). Production of ^{15}N products sometimes increased with time after $^{15}NO_3^-$ addition (Figs. 4 & 5c,d). At Stn B6, N_2 gas ($^{29+30}N_2$) flux increased significantly on Day 6 versus Day 3 (Fig. 4c). At Stns L155 and L189, $^{15}NH_4^+$ flux increased significantly on Day 3 versus Day 2 of the incubation (Fig. 5d). Except for these incidents, the increases in fluxes over time were not significant (Figs. 4 & 5c,d).

Flux changes in the different forms of N_2 gas and NO_3^- and NH_4^+ before and after the $^{15}NO_3^-$ addition are

presented in Table 2. The denitrification rate based on the $^{14}NO_3^-$ ($^{28}N_2$ production; before $^{15}NO_3^-$ addition) was higher in August than in December 1999. Negative $^{28}N_2$ flux (flux into the sediment) was observed in December 1999, suggesting N fixation. However, the rate was significantly different from zero only at Laguna Madre stations ($p < 0.05$). The Baffin Bay stations had higher $^{28}N_2$ production than Laguna Madre in August and December 1999, but the difference was not significant.

In August 1999, 'total' denitrification activity ($^{28}N_2$ plus $^{29+30}N_2$) did not increase significantly after the $^{15}NO_3^-$ addition ($p > 0.05$). Total denitrification rates increased after the addition in December 99, but the amounts were small (16 and 26 µmol m^{-2} h^{-1} in Baffin Bay and Laguna Madre, respectively) and the increase was not significant in Baffin Bay ($p > 0.05$). $^{15}NH_4^+$ fluxes after the addition ranged from 23 to 79 µmol m^{-2} h^{-1} (Table 2). Laguna Madre had a higher $^{15}NH_4^+$ flux than Baffin Bay. A seasonal trend was not obvious in Laguna Madre while the $^{15}NH_4^+$ flux in Baffin Bay was higher in August 1999 compared to December 1999. In December 1999, a small negative $^{15}NH_4^+$ flux was observed before the addition, probably due to analytical variability of the HPLC system (Gardner et al. 1995).

Before the addition, a small NO_3^- flux out of the sediments was observed at all stations during both seasons. After the addition, NO_3^- fluxes into the sediment were high (up to 370 µmol m^{-2} h^{-1}). Baffin Bay sediments consumed more NO_3^- (higher negative NO_3^- flux) than Laguna Madre sediments after the addition, but the difference between the 2 sites was smaller in December 1999. Since NO_3^- fluxes were small before the addition, all NO_3^- flux after the addition was assumed to be $^{15}NO_3^-$ for comparison with $^{29+30}N_2$ and $^{15}NH_4^+$ fluxes. Denitrification consumed 5 to 29% of the added $^{15}NO_3^-$ while DNRA accounted for 15 to 75% of the added $^{15}NO_3^-$ (Table 2). Both $^{29+30}N_2$ and $^{15}NH_4^+$ fluxes were smaller in Baffin Bay than Laguna Madre, whereas NO_3^- flux was higher in Baffin Bay. As a result, the percentage recovery of added $^{15}NO_3^-$ was higher in Laguna Madre than Baffin Bay. The recovery of $^{15}NO_3^-$ as $^{29+30}N_2$ and $^{15}NH_4^+$ did not show seasonal differences. For example, $^{15}NH_4^+$ flux remained almost the same in December as in August 1999. The total recovery of added $^{15}NO_3^-$ as $^{29+30}N_2$ and $^{15}NH_4^+$ ranged from 20 to 91%, but seasonal differences were not noticeable.

DISCUSSION

Denitrification rates in Baffin Bay (0 to 68 µmol m^{-2} h^{-1}) and Laguna Madre (0 to 41 µmol m^{-2} h^{-1}) are in the range of those reported for other coastal marine environments (Seitzinger 1988, Herbert 1999) but lower than those for other Texas estuaries, such as Galveston Bay (~170 µmol m^{-2} h^{-1}, using direct N_2 flux method; An & Joye 2001). Denitrification rates measured in Galveston Bay with the N_2 flux method after gas purging (0 to 47 µmol m^{-2} h^{-1}; Zimmerman & Benner 1994) were comparable to our measurements although the gas purging denitrification method is problematic because of extended incubation times required to remove atmospheric N_2 (see reviews in An & Joye 1997, Cornwell et al. 1999). Results from other denitrification measurements using the gas purging N_2 flux method in Texas estuaries are also similar to the rates of this study (4 to 71 and 4.6 to 35 µmol m^{-2} h^{-1} for Nueces and Guadalupe estuaries, respectively; Yoon & Benner 1992).

In April 1999, denitrification rates were measured in Laguna Madre and Baffin Bay using the same experimental setup as in the current study without $^{15}NO_3^-$ enrichment (An & Gardner 2000). Mean denitrification rates observed in April 1999 (~130 µmol m^{-2} h^{-1}; An & Gardner 2000) were higher than in August 1999 (41 to 77 µmol m^{-2} h^{-1}; current study) despite the lower temperature. SOD was also higher in spring (~1500 µmol m^{-2} h^{-1}; An & Gardner 2000) than in August 1999 (964 µmol m^{-2} h^{-1}; Current study). Low SOD in August 1999 may have resulted from organic matter limitation. Water column chl *a* concentration was highest in April 1999 and decreased in August and December 1999 in Laguna Madre/Baffin Bay (T. Villareal unpubl. data), suggesting a tight benthic-pelagic coupling. Low concentrations of available organic carbon would limit heterotrophic activity even under favorable temperatures. Since denitrification is a heterotrophic process, low organic matter availability could also limit denitrification rates (Koike & Sørensen 1988, Cornwell et al. 1999). Another explanation for the low summertime denitrification rates could be sulfide inhibition (Gould & McCready 1982, Jensen & Cox 1992, Joye & Hollibaugh 1995). Anoxic regeneration would increase as oxygen is consumed. In a saline estuary like Laguna Madre/Baffin Bay (salinity during this study = 25 to 38 ppt), sulfate reduction is a major anoxic process and sulfide (the product of sulfate reduction) concentrations in porewater may increase under low oxygen conditions (Morse et al. 1992). Sulfide is toxic to many sediment bacterial processes including nitrification and denitrification (Gould & McCready 1982, Jensen & Cox 1992, Joye & Hollibaugh 1995). Due to the shallow water depth, the water column was well mixed, even in August 1999 (Table 1), but oxygen penetration-depth of the sediment may have decreased during summer at times when conditions were stable. Although porewater sulfide concentrations were not measured in the current study area, high sulfate reduction activity near

the sediment water interface (43 to 112 mmol l^{-1} yr^{-1} in June 1988; Morse et al. 1992) and high porewater sulfide concentrations (up to 5500 µM in June 1988, Morse et al. 1992; 102 to 160 µM in July 1996, Lee & Dunton 2000) occur in this region. It is also possible that salinity-induced inhibition of denitrifying activity by physiological effects other than sulfide inhibition could explain the low denitrification activity in saline estuaries like Baffin Bay and Laguna Madre (Rysgaard et al. 1999). Additionally, $^{15}NO_3^-$ may have converted to $^{15}N_2O$ by incomplete denitrification in a high sulfide environment (Brundet & Garcia-Gil 1996). Although the production of $^{15}N_2O$ was not quantitatively measured, $^{15}N_2O$ signals (amu 46) were within background levels, suggesting that large amounts of $^{15}N_2O$ were not produced during the measurement.

In winter, denitrification rates were not significantly different from zero, and N_2 flux into the sediment due to N_2 fixation was visible in Laguna Madre. N_2 flux in Laguna Madre (20 µmol m^{-2} h^{-1}) may provide a conservative estimate of net N_2 fixation in this area because our incubation was done under dark or low light conditions (Joye & Paerl 1994, An et al. 2001). The measured rate is at the low end of the range reported for other seagrass beds. Typically, seagrass beds have higher N_2 fixation rates (1 to 250 µmol m^{-2} h^{-1}) than uncolonized areas (0.02 to 5 µmol m^{-2} h^{-1}; Herbert 1999).

Total denitrification rates ($^{28}N_2$ + $^{29+30}N_2$) were not enhanced significantly by $^{15}NO_3^-$ additions. This result was surprising because in many estuaries denitrification is limited by NO_3^- availability (Koike & Sørensen 1988, Cornwell et al. 1999). Instead, a portion of the added $^{15}NO_3^-$ was converted to $^{15}NH_4^+$. The $^{15}NH_4^+$ production rate ranged from about 20 to 80 µmol m^{-2} h^{-1} after the $^{15}NO_3^-$ addition. Although the $^{15}NO_3^-$ enrichment level varied (~15 to 30 µM and ~100 µM in Koike & Hattori [1978] and this study, respectively) and the experimental setup was different, $^{15}NH_4^+$ production rates were comparable to those measured in Koike & Hattori (1978; 5 to 228 µmol m^{-2} h^{-1}, assuming 0 to 3 cm depth integration and 2.3 g cm^{-3} bulk sediment density). Higher production rates (800 to 3200 µmol m^{-2} h^{-1}) were observed with a high $^{15}NO_3^-$ enrichment (~700 µm, assuming 0 to 4 cm depth integration; Jørgensen 1989). Our rates were much lower than those from a sediment slurry incubation (~2300 µmol m^{-2} h^{-1}; assuming 0 to 12 cm depth integration; Sørensen 1978) with a similar $^{15}NO_3^-$ enrichment (~100 µM). Reducing conditions favor DNRA over denitrification, and slurry experiments tend to have higher DNRA rates compared to intact core experiments (Kasper 1983). In our study, DNRA rates were higher than denitrification rates even though intact cores were used for incubation and O_2 concentration in the water column was maintained at more than 80% of saturation. Thus, it seems unlikely that this high DNRA partitioning over denitrification resulted from reduced conditions in the sediments as suggested by Kasper (1983). We do not believe that the high partitioning of NO_3^- to DNRA was a result of $^{15}NO_3^-$ enrichment. NO_3^- enrichment should not only stimulate denitrification and DNRA but can be expected to favor denitrification over DNRA (Sørensen 1987). Additionally, since DNRA bacteria have a higher K_m (half saturation concentration, 100 to 500 µM NO_3^-; Jørgensen 1989) than denitrifiers (5 to 10 µM NO_3^-; Jørgensen 1989), the enrichment level in our study (~100 µM) should firstly favor denitrification rather than DNRA if denitrification was limited by NO_3^- availability.

Low denitrification and high DNRA were observed during summer in a French coastal lagoon (Rysgaard et al. 1996). Sulfate reduction was high and low denitrification rates were attributed to inhibited nitrification. High DNRA was attributed to the presence of sulfate reducing bacteria, which have DNRA capacity as a secondary metabolism (Rysgaard et al. 1996). This observation agrees with those of King & Nedwell (1985) and Jørgensen (1989). DNRA may be favored in environments where NO_3^- availability is variable because DNRA bacteria have constitutive enzymes (Jørgensen 1989).

Chemolithoautotrophic bacteria that use reduced sulfur compounds as an electron donor and reduce NO_3^- to NH_4^+ have been reported (Schedel & Truper 1980), but the ecological significance of the process is not known (Sørensen 1987). NH_4^+ production rates coincided with NO_3^- and H_2S depletion rates in sediment slurry experiments enriched with NO_3^- and different forms of sulfur compounds (Brundet & Garcia-Gil 1996). Whereas denitrification was incomplete (the final product was N_2O instead of N_2), DNRA occurred in the presence of high sulfide concentrations. In contrast, denitrification was the major NO_3^- sink when sulfide concentrations were low (Brundet & Garcia-Gil 1996). Abundant populations of NO_3^--storing sulfur bacteria of the genera *Thioploca*, *Beggiatoa* and *Thiomargarita* have been reported in sediments of coastal upwelling regions (Schulz et al. 1999, Graco et al. 2001). A chemolithotrophic coupling of NO_3^- and sulfide through NO_3^--storing sulfur bacteria may be a widespread feature of coastal sediments (Schulz et al. 1999).

Along with the high sulfide concentrations that occur in this region (Lee & Dunton in press), our data support the idea that sulfide could influence DNRA at both of our sampling sites. Being a negative estuary where evaporation exceeds precipitation, salinity in Laguna Madre/Baffin Bay is high and sulfate reduction may be a major organic-matter degradation process. Higher DNRA observed in Laguna Madre versus Baffin Bay

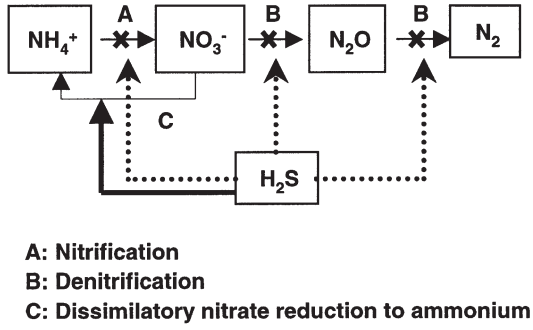

Fig. 6. Proposed relationship between sulfide and nitrogen transformations. Solid arrows represent a positive effects and broken arrows represent a negative effects. Modified from Brunet & Garcia-Gil (1996)

(Table 2) could result from high sulfide reduction activity fueled by seagrass detritus (Rysgaard 1996, Eldridge & Morse et al. 2000, Graco et al. 2001). Fig. 6 shows how sulfide may influence N transformations in this system. High sulfide concentrations may inhibit nitrification and keep NO_3^- availability low. Since denitrification is inhibited by sulfide, any NO_3^- that is present will be available for DNRA rather than denitrification. Inhibition of denitrification can explain the enhanced DNRA that we observed, even though the K_m for DNRA bacteria is higher than for denitrifiers, and $^{15}NO_3^-$ enrichment was low. Although high sulfide concentrations inhibit nitrification and denitrification, sulfide may fuel DNRA by providing an electron donor. High SOD, high DNRA and low denitrification rates in Laguna Madre compared to Baffin Bay support this hypothesis.

The recovery of $^{15}NO_3^-$ as $^{15}NH_4^+$ and $^{29+30}N_2$ was higher in Laguna Madre than Baffin Bay (Table 2). $^{15}NO_3^-$ flux into the sediment was higher in Baffin Bay while $^{15}NH_4^+$ and $^{29+30}N_2$ flux was lower than in Laguna Madre, which caused low $^{15}NO_3^-$ recovery in Baffin Bay. The $^{15}NH_4^+$ flux difference was more distinctive than the $^{29+30}N_2$ flux. Possible explanations are differences in sediment types between the 2 sites and/or the presence of seagrasses in Laguna Madre but not in Baffin Bay. The abundance and community structure of microbes in the seagrass bed may be different than those regions without seagrass. For example, bacteria responsible for $^{15}NH_4^+$ production in Laguna Madre may be sulfate reducers that have NO_3^- reduction capability as a second metabolism (Rysgaard et al. 1996).

The ^{15}N content in organic matter was not measured in our study but it is reasonable to speculate that a loss to organic N production could account for some of the lost $^{15}NO_3^-$, even though the proportion may be small (Jørgensen 1989; 2 to 4%). Our average recovery of 56% was similar to that in Norsminde Fjord, Denmark (Jørgensen 1989). However, the proportion of DNRA (recovered as $^{15}NH_4^+$) was higher (42%) than values reported by Jørgensen (1989; 22%). Recovery via denitrification in our study was 14%, which is lower than the Jørgensen (24%; 1989) or Kasper values (70 to 95%; 1983).

Sulfide-inhibited denitrification and sulfide-induced DNRA would contribute to the preservation of N in Laguna Madre/Baffin Bay. Preserved N may sustain biota in this region when other nutrient inputs are absent. It also may explain apparent phosphate limitation of primary production observed at times in the region (Dr. J. Cotner, University of Minnesota, pers. comm.). High DNRA potential in this region suggests that the dominant form of inorganic N available to phytoplankton is NH_4^+ rather than NO_3^-. The Texas brown tide organism, *Aureomonas lagunensis*, which can use NH_4^+ or NO_2^-, but cannot use NO_3^- as a nitrogen source, could outcompete other algae in this NH_4^+ replete environment. It is reasonable to suggest that the persistency of the Brown Tide algal bloom in this area may have been enhanced by DNRA activity.

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