Biogeochemistry (2009) 95:185–198
DOI 10.1007/s10533-009-9329-5

Nitrogen dynamics at the sediment–water interface in shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with increased eutrophication

Wayne S. Gardner · Mark J. McCarthy

Received: 19 August 2008 / Accepted: 29 April 2009 / Published online: 26 May 2009

Abstract Nitrogen (N) dynamics at the sediment–water interface were examined in four regions of Florida Bay to provide mechanistic information on the fate and effects of increased N inputs to shallow, subtropical, coastal environments. Dissimilatory nitrate (NO_3^-) reduction to ammonium (DNRA) was hypothesized to be a significant mechanism retaining bioreactive N in this warm, saline coastal ecosystem. Nitrogen dynamics, phosphorus (P) fluxes, and sediment oxygen demand (SOD) were measured in north-central (Rankin Key; eutrophic), north-eastern (Duck Key; high N to P seston ratios), north-western (Murray Key; low N to P ratios), and central (Rabbit Key; typical central site) Florida Bay in August 2004, January 2005, and November 2006. Site water was passed over intact sediment cores, and changes in oxygen (O_2), phosphate ($o\text{-}PO_4^{3-}$), ammonium (NH_4^+), NO_3^-, nitrite (NO_2^-), and N_2 concentrations were measured, without and with addition of excess $^{15}NO_3^-$ or $^{15}NH_4^+$ to inflow water. These incubations provided estimates of SOD, nutrient fluxes, N_2 production, and potential DNRA rates. Denitrification rates were lowest in summer, when SOD was highest. DNRA rates and NH_4^+ fluxes were high in summer at the eutrophic Rankin site, when denitrification rates were low and almost no N_2 came from added $^{15}NO_3^-$. Highest $^{15}NH_4^+$ accumulation, resulting from DNRA, occurred at Rabbit Key during a picocyanobacteria bloom in November. $^{15}NH_4^+$ accumulation rates among the stations correlated with SOD in August and January, but not in November during the algal bloom. These mechanistic results help explain why bioreactive N supply rates are sometimes high in Florida Bay and why denitrification efficiency may decrease with increased NO_3^- inputs in sub-tropical coastal environments.

Keywords Florida Bay · Nitrogen transformations · DNRA · Denitrification · Subtropical/tropical ecosystems

Introduction

An improved understanding of nitrogen (N) transformations and fates on local and global scales is needed to protect the water quality and health of aquatic ecosystems as N inputs increase with increased fertilizer production and use (Burgin and Hamilton 2007; Galloway et al. 2008; Horner-Devine and Martiny 2008; Mulholland et al. 2008; Seitzinger 2008). One major research question that has been

---

W. S. Gardner (✉)
The University of Texas at Austin Marine Science Institute, Port Aransas, Austin, TX 78373, USA
e-mail: wayne.gardner@mail.utexas.edu

Present Address:
M. J. McCarthy
Département des sciences biologiques, Université du Québec à Montréal, CP 8888, Succarsale Centre-Ville, Montréal, QC H3C 3P8, Canada

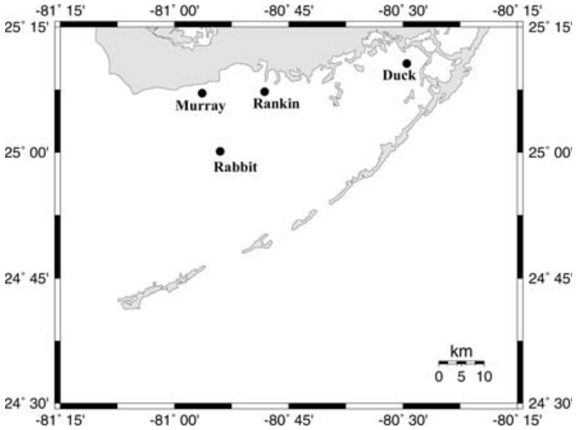

Fig. 1 Location of sampling stations in Florida Bay

identified is: "How will tropical regions respond to rising N inputs?" (Galloway et al. 2008). The effects of N inputs into coastal systems are compounded by microbial N transformations involving nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), dinitrogen (N_2), or organic N, which in turn influence the composition of primary and secondary producers.

Florida Bay is a shallow, saline, subtropical bay located south of the Florida mainland and west of the Florida Keys (Fig. 1). It is divided into discrete shallow-water ecosystems by shallow carbonate mud banks, which restrict water exchange (Lavrentyev et al. 1998; Boyer et al. 1999; Fourqurean and Robblee 1999; Phlips et al. 1999). These sub-basins range from eutrophic to those less affected by nutrient inputs, with varying seston nutrient ratios (Lavrentyev et al. 1998; Cotner et al. 2000). The western zone, represented in this paper by Murray Key, is most affected by Gulf of Mexico waters and characterized by near or below Redfield seston N:P ratios. The north-central zone, represented by Rankin Key, has high N:P ratios and is eutrophic, likely due to nutrient inputs from southern Florida (Lavrentyev et al. 1998; Lapointe and Barile 2004). The eastern zone, represented by Duck Key, has variable salinity, high dissolved inorganic N (DIN) concentrations, high N:P ratios for seston, and low productivity (Boyer et al. 1997; Lavrentyev et al. 1998; Fourqurean and Robblee 1999). The central zone (Rabbit Key) has intermediate conditions and is considered a "typical" Florida Bay site.

Florida Bay has been affected by blooms of the picoplanktonic cyanobacterium, *Synechococcus elongatus* (Phlips et al. 1999), and the concurrent death of seagrasses (e.g., *Thalassia testudinum*), which are nursery grounds for valuable organisms in the bay (Robblee et al. 1991). Reasons for these detrimental trends are not clear, but high salinities (Zieman et al. 1999), sulfide toxicity (Koch and Erskine 2001), and/or increased nutrient loadings (Lapointe and Clark 1992) were considered as contrasting potential factors driving ecosystem changes and seagrass die-offs in Florida Bay.

Florida Bay differs from many marine systems in that N is replete in some areas, and P, rather than N, often limits biological productivity, especially in the eastern region (Fourqurean et al. 1993; Phlips and Badylak 1996; Lavrentyev et al. 1998; Cotner et al. 2000). Also, reduced inorganic N forms (NH_4^+ and NO_2^-) are often as or more abundant than NO_3^- (Lavrentyev et al. 1998; Boyer et al. 1999; Cotner et al. 2000; this study). High NH_4^+ (Phlips et al. 1995) and organic N (Glibert et al. 2004) availability may promote harmful algal blooms, with subsequent losses of sea grass beds from shallow regions. Dissimilatory NO_3^- reduction processes, such as denitrification (Seitzinger 1988; Cornwell et al. 1999), dissimilatory NO_3^- reduction to NH_4^+ (DNRA; Koike and Hattori 1978; Sørensen 1978), and dissimilatory NO_3^- reduction to NO_2^- (DNRN; Kelso et al. 1999), may have significant effects on sediment–water interface N and oxygen dynamics in the bay. The end-product of denitrification and anaerobic NH_4^+ oxidation (anammox) is N_2, a bioreactive-N "sink". In anammox, NO_2^- and NH_4^+ are converted biologically into N_2 by the catabolic reaction, $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (Dalsgaard and Thamdrup 2002, Kuypers et al. 2003) rather than by heterotrophic reduction of NO_3^-, as occurs in denitrification (Seitzinger 1988). DNRA is a heterotrophic reaction, which usually occurs under low redox conditions with NO_2^- as an intermediate product (Tiedje 1988; Rysgaard et al. 1996; An and Gardner 2002). It is enhanced by sulfate-reducing bacteria (Rysgaard et al. 1996), whereas nitrification (Joye and Hollibaugh 1995) and the final step of denitrification ($N_2O \rightarrow N_2$; Brunet and Garcia-Gil 1996) are inhibited by sulfide. DNRA and DNRN can serve as bioreactive-N "links" in ecosystems (An and Gardner 2002) but are often ignored in considerations of coastal eutrophication (Bianchi et al. 1999).

Differing nutrient conditions among regions makes Florida Bay an interesting subtropical ecosystem to study the effects of nutrient status and other factors on coastal N- and oxygen-dynamics, such as DNRA, denitrification, and anammox, which are not well understood in tropical and subtropical coastal environments (Galloway et al. 2008). For example, why does denitrification efficiency decrease with increasing NO_3^- concentrations and respiration rates (Mulholland et al. 2008; Seitzinger 2008)? Active DNRA, observed in warm, hypersaline, subtropical Texas coastal systems (An and Gardner 2002; Gardner et al. 2006) and other estuarine environments (summarized by Megonigal et al. 2004), led to our central hypothesis: *DNRA is a significant process retaining bioavailable, fixed N in Florida Bay by producing* NH_4^+ *as a dissimilatory end product of* NO_3^- *reduction.* In this paper, we consider this hypothesis and related questions regarding N and O_2 dynamics at the sediment–water interface in subtropical coastal environments, including: (*1*) *What is the relative importance of DNRA versus denitrification* (*or anammox*) *in controlling the fate of bio-reactive N?* (*2*) *Is* NO_3^- *from overlying water reduced to* N_2 *or* NH_4^+ *as efficiently as that formed by in situ nitrification?* (*3*) *How do rates of these key processes relate to season, sampling site, and eutrophication status?* These questions were addressed by measuring SOD and N transformations and fluxes using continuous-flow experiments on intact cores from the four representative regions in August 2004, January 2005, and November 2006.

Experimental sites and methods

Field sampling and experiments were conducted using research vessels and laboratory facilities at the Keys Marine Laboratory (KML), Long Key, Florida. Water samples and triplicate (Aug 2004 and Jan 2005) or duplicate (Nov 2006) intact cores were collected at each of the four sites (Fig. 1) representing distinct Florida Bay regions. Water temperature, depth, salinity, O_2 concentration, and chlorophyll fluorescence were measured at each site using a Hydrolab Data Sonde/Surveyor 4. Water samples collected for nutrient analyses were filtered (0.2 µm pore size syringe filter) on-site and frozen until analysis. Concentrations of *o*-PO_4^{3-}, $NO_3^- + NO_2^-$, and NO_2^- were measured with a Lachat Quikchem 8000 Flow Injection Analysis system. The methods were evaluated for salinity effects on concentration results by creating calibration curves at salinities of 0, 5, 20, and 50. A linear, salinity-related over-estimation of NO_2^- was observed, and NO_2^- data were corrected by inserting sample salinity into the linear regression equation to determine the corrected NO_2^- concentration. Nitrate concentration was then determined by subtracting the corrected NO_2^- concentration from the initial $NO_3^- + NO_2^-$ concentration. Ammonium concentrations were measured by high performance liquid chromatography (HPLC; Gardner et al. 1995).

Net nutrient fluxes, denitrification, and potential DNRA and anammox rates at the sediment–water interface were measured on intact sediment cores (An et al. 2001; 7.6 cm diameter, ca. 10–15 cm depth; 3 per station in August and January; 6 per station in November) collected from a small boat using a coring device equipped with a core cylinder, a PVC pipe handle, and a one-way check valve to preserve core and overlying water integrity (Gardner et al. 2006).

After return to KML, each intact core was installed into a continuous-flow system consisting of an intake water vessel, flow tubes, intact sediment core, peristaltic pump, incubation bath, and sample collection vessel (Lavrentyev et al. 2000; An et al. 2001). A plunger, with teflon inlet and outlet tubes and sealed to the core cylinder with an o-ring, was installed over each sediment core and adjusted to ~5 cm above the sediment surface to leave an overlying water volume of about 230 mL. Each core was incubated at near in situ temperature and under laboratory light conditions, and site bottom water was passed over the core surface continuously at 1.5 mL min^{-1}. After one day was allowed for the systems to approach steady-state conditions, inflow and outflow samples were collected daily for dissolved gas and nutrient analyses. Samples for dissolved gas analysis were preserved with 200 µL 50% $ZnCl_2$ solution, stored underwater, and transported to the University of Texas Marine Science Institute (UTMSI) for measurement of net N_2 and O_2 fluxes by membrane inlet mass spectrometry (MIMS; Kana et al. 1994; An et al. 2001; McCarthy et al. 2007). Nutrient samples were filtered (0.2 µm pore-size syringe filters) in the laboratory and frozen for analysis as described above. Sediment–water interface gas and nutrient fluxes were calculated from concentration differences between inflow and outflow samples, flow rates, and cross-sectional area of the cores (Lavrentyev et al. 2000).

Two sequential sets of experiments at all four sites were conducted during each field trip. In August, an interval of 1 week occurred between the first and second experiments, and the results were treated and presented as separate experiments (Figs. 2–4). For August and the first January experiment, inflow water was spiked with excess (20–40 µM final concentration) $^{15}NO_3^-$ after 2 days of sampling, and the flow was continued overnight to allow the chambers to approach steady-state. Inflow and outflow waters were sampled for gas and nutrient analyses for two more days. In January, experiment 2 was modified to spike the inflow water with $^{15}NH_4^+$ (ca. 5 µM), instead of $^{15}NO_3^-$, to evaluate the possible presence and importance of anammox. In November 2006, the protocol was changed to spiking duplicate cores with $^{15}NH_4^+$ or $^{15}NO_3^-$ at the beginning of the experiments and then comparing results, after steady-state conditions were reached, to those of duplicate controls, which were not spiked. These CAN (control, ammonium, nitrate) experiments thus evaluated potential anammox and offered the advantage of shorter total incubation times to examine N and O_2 dynamics. Concentrations of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, and Ar were measured with MIMS to differentiate denitrification of internal sources of N versus that derived from added $^{15}NO_3^-$ (An et al. 2001) or $^{15}NH_4^-$ in the overflowing water. Total NH_4^+ and $^{15}NH_4^+$ (potential DNRA) production rates were measured by HPLC (Gardner et al. 1995; An et al. 2001).

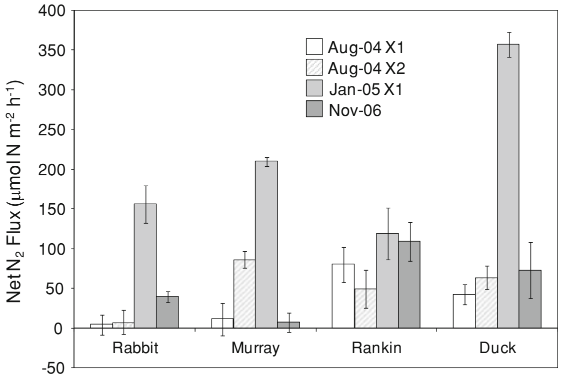

Fig. 2 Net N_2 fluxes in August 2004, January 2005, and November 2006 experiments without addition of labeled N compounds to inflow waters. Error bars reflect one standard error

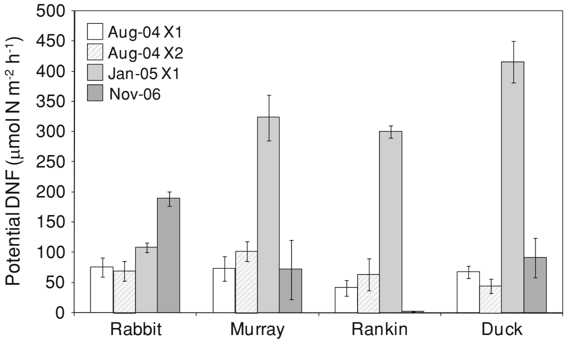

Fig. 3 Potential denitrification rates (i.e., positive N_2 fluxes) in August 2004, January 2005, and November 2006 experiments after the addition of 20–40 µM $^{15}NO_3^-$ to inflow waters. Error bars reflect one standard error

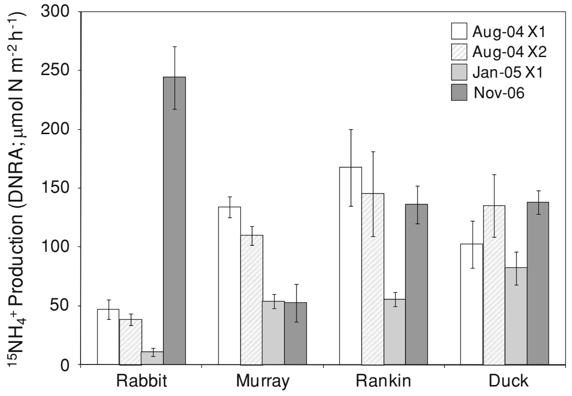

Fig. 4 Potential DNRA rates at 4 stations in Florida Bay in August 2004, January 2005, and November 2006. Error bars reflect one standard error

High-level additions of the isotopic forms of N were preferred over low-level tracer additions (e.g., <10% ambient concentrations) because our analytical methodologies (MIMS and HPLC) required sufficient concentrations and ratios of labeled ^{15}N compounds for measurement of significant changes in the different N forms during the time scale of the continuous-flow experiments. Also, the ambient concentrations of the N compounds, often about 1 µM N, were low relative to their fluxes of up to hundreds of µmol N m^{-2} h^{-1}, even before isotope addition in the case of NH_4^+ (see results). Finally, the additions of higher than ambient levels of labeled substrates to the water flowing over the cores provided information about the potential fate of N compounds occurring in the water, as would occur with episodic tributary inputs of nutrients.

Table 1 (Taken from McCarthy et al. 2009). In situ water column characteristics in Florida Bay

Station	Date	Depth (m)	Sal	Temp (°C)	PAR (%)	DO (mg l^{-1})	chl *a* (µg l^{-1})	NH_4^+ (µM)	NO_3^- (µM)	NO_2^- (µM)	*o*-PO_4^{3-} (µM)
Rabbit	Aug-2004	0.81	38.4	31.6	35	5.2	0.6	0.49	0.93	0.57	0.11
	Jan-2005	0.95	38.3	23.0	36	4.6	1.5	0.61	0.89	0.38	0.04
	Nov-2006	0.72	36.9	25.8	26	6.1	7.9	BDL	0.33	0.45	0.17
Murray	Aug-2004	0.38	37.2	31.6	36	6.6	1.4	BDL	1.00	0.61	0.08
	Jan-2005	1.24	33.7	23.5	17	5.1	4.3	0.35	0.63	0.29	0.04
	Nov-2006	0.83	35.7	25.2	11	5.5	4.4	BDL	0.50	0.54	0.06
Rankin	Aug-2004	0.18	40.2	31.8	59	4.7	1.7	1.49	1.08	0.61	0.06
	Jan-2005	1.03	45.1	23.1	32	4.7	2.9	0.44	1.12	0.37	0.02
	Nov-2006	0.74	31.7	25.3	7	7.4	7.0	BDL	0.25	0.42	0.12
Duck	Aug-2004	0.93	41.5	31.1	49	5.1	0.0	1.17	1.18	0.58	0.10
	Jan-2005	0.74	41.2	22.9	37	5.3	1.9	4.43	1.85	0.43	0.04
	Nov-2006	0.78	31.4	24.9	30	5.0	1.6	0.42	0.28	0.45	0.05

Values for August 2004 and January 2005 represent an average of two visits to each site. *Sal*, salinity; *Temp*, temperature; *PAR*, photosynthetically active radiation and is presented as the percentage of PAR at the water surface that reaches the sediment surface; *DO*, dissolved oxygen. *Chl*, chlorophyll; *BDL*, below detection limit

The sequentially-measured fluxes within a specific treatment for each core were averaged to provide an individual mean flux for that core treatment. The resulting individual values were averaged for replicate core treatments to produce mean fluxes ± one standard error (SE), which in turn were used to compare rates among treatments and sampling events. For the purposes of this study, the detection limits for the respective flux measurements were defined as the values where the SE's did not overlap with zero. Rates from isotopic enrichments that were not different from control rates (ANOVA comparison) were considered as not detectable.

Results

Station characteristics and nutrient concentrations in August 2004, January 2005, and November 2006

Site water depths on the different dates ranged from 0.2 to 1.2 m, and salinities ranged from 31 to 45 (Table 1; from McCarthy et al. 2009). Temperatures were 31 and 32°C in August, ca. 23°C in January, and 25 and 26°C in November. Ammonium concentrations ranged from below detection to 1.5 µM at all stations, except for a high value of 4.4 µM at Duck in January. Nitrate concentrations ranged from 0.3 to 1.8 µM with the most extreme values at Duck. Nitrite concentrations showed intermediate concentrations of 0.3 to 0.6 µM at all stations and were generally comparable to NO_3^- and NH_4^+ concentrations.

Nutrient fluxes

Nitrogen fluxes (Tables 2, 3, 4) are expressed as µmol N m^{-2} h^{-1}. Positive rates indicate flux into the water, whereas negative rates indicate removal as the water passed over the intact sediment cores. Phosphate fluxes ranged from 0.1 to 13 µmol P m^{-2} h^{-1}. Mean NH_4^+ fluxes were positive at all stations before $^{15}NO_3^-$ addition, with rates ranging from 60 to 540 in August, 7 to 310 in January, and 31 to 380 in November. Nitrate fluxes without isotope addition ranged from −1 to 2 in August, −2 to 2 in January, and −0.1 to 0.6 in November. Thus, NH_4^+ fluxes were relatively high and variable, whereas NO_3^- and NO_2^- net fluxes remained near zero and showed little variation.

After the $^{15}NO_3^-$ additions, NO_3^- removal rates and NH_4^+ and NO_2^- production rates increased substantially at all stations in August, January, and November (Tables 2, 3, 4). Nitrate fluxes after additions ranged from −12 to −150 in August, +13 to −190 in January, and −2 to −720 in November. Corresponding NH_4^+ fluxes ranged from 170 to 620 in August, 60 to 560 in January, and 180 to 770 in

Table 2 Sediment–water nutrient fluxes (μmol m^{-2} h^{-1}) plus/minus one standard error (SE) in August 2004 experiments before and after $^{15}NO_3^-$ addition (20–40 μM) to inflow waters

	Rabbit		Murray		Rankin		Duck	
Experiment #	1	2	1	2	1	2	1	2
NH_4^+ before	60	100	160	170	340	540	130	250
SE	13	24	47	63	100	120	35	91
NH_4^+ after	170	210	445	540	580	620	280	520
SE	16	43	34	40	100	73	44	100
NO_3^- before	41	1.4	4.0	−1.6	1.7	0.1	0.1	4.0
SE	28	0.5	1.4	1.1	2.0	1.3	1.6	1.7
NO_3^- after	−12	−54	−138	−150	−138	−88	−118	−134
SE	15	14	19	7.7	19	16	23	13
NO_2^- before	1.9	−1.1	0.0	−1.2	0.3	0.6	−1.0	−0.9
SE	2.5	0.9	0.7	1.8	0.7	0.3	0.6	0.8
NO_2^- after	10	5.1	16	23	43	52	19	32
SE	2.0	1.8	2.3	2.3	12	18	6.5	10
PO_4^{3-} before	6.7	3.4	1.5	4.2	0.5	2.2	1.9	2.2
SE	4.6	4.4	0.6	0.8	0.1	0.2	0.9	0.4
PO_4^{3-} after	0.2	0.7	2.7	6.0	1.3	2.1	0.6	3.0
SE	0.1	0.2	0.6	0.6	0.5	0.2	0.3	0.6

Positive values indicate fluxes into the water from the sediments. Negative values indicate fluxes into sediments

Table 3 Sediment–water nutrient fluxes (μmol m^{-2} h^{-1}) in January 2005 experiments before and after $^{15}NO_3^-$ (20–40 μM; experiment 1) or NH_4^+ (ca. 5 μM; experiment 2)

	Rabbit		Murray		Rankin		Duck	
Experiment #	1	2	1	2	1	2	1	2
NH_4^+ before	40	230	170	41	29	6.9	220	310
SE	13	11	29	4.0	11	3.4	110	140
NH_4^+ after	73	250	330	120	200	61	560	440
SE	22	35	22	16	14	39	250	110
NO_3^- before	0.6	3.6	1.5	−2.1	2.9	−0.6	−6.6	−13
SE	1.1	0.7	1.0	1.5	1.7	0.5	2.5	2.4
NO_3^- after	−30	0.6	−5.8	1.1	−21	1.2	−190	13
SE	23	0.9	31	0.7	29	1.2	35	1.7
NO_2^- before	−0.4	1.8	0.9	2.2	0.7	0	0.4	−1.7
SE	0.7	1.0	0.2	0.3	0.4	0.2	0.4	0.5
NO_2^- after	0	0.5	1.6	0	0	−0.2	7.3	−1.5
SE	0	0.5	0.8	0.2	0	0.3	4.0	0.4
PO_4^{3-} before	1.2	1.6	5.8	1.4	0.2	0.3	12.6	10
SE	1.0	1.5	2.9	0.3	0.1	0.1	6.4	5.0
PO_4^{3-} after	0.5	0.2	6.5	1.5	0.4	0.5	4.7	6.1
SE	0.1	0.1	1.5	0.4	0.1	0.2	2.4	3.4

Positive values indicate fluxes into the water from the sediments. Negative values indicate fluxes into sediments

Table 4 Sediment—water nutrient fluxes (μmol m^{-2} h^{-1}) in November 2006 experiments without and with $^{15}NO_3^-$ (20–40 μM) or ^{15} NH_4^+ (5 μM) additions

	Rabbit			Murray			Rankin			Duck		
Treatment	C	NH_4^+	NO_3^-	C	NH_4^+	NO_3^-	C	NH_4^+	NO_3^-	C	NH_4^+	NO_3^-
NH_4^+	140	470	770	31	67	180	78	96	420	380	180	410
SE	65	120	37	8.0	58	46	20	10	49	120	24	18
NO_3^-	4.3	3.9	−720	1.1	2.5	−450	2.1	−0.1	−290	−2.0	−0.1	−210
SE	1.4	0.7	51	1.6	2.0	130	0.8	0.4	48	0.8	0.51	70
NO_2^-	−0.1	−0.6	36	0.2	0.3	30	0.6	0.02	23	0.3	−0.5	27
SE	0.3	0.2	2.6	0.3	0.5	9.7	0.2	0.3	6.3	0.3	0.3	6.4
PO_4^{3-}	0.1	0.5	0.4	0.7	0.7	0.5	0.3	0.3	−0.5	0.5	0.2	1.1
SE	0.1	0.1	0.04	0.1	0.2	0.04	0.1	0.1	0.2	0.2	0.1	0.7

Positive values indicate fluxes into the water from the sediments. Negative values indicate fluxes into sediments. *C*, Control without ^{15}N-additions

November. Nitrite fluxes after additions were 5 to 52 in August, −1.5 to 7 in January, and 23 to 30 in November (Tables 2, 3, 4). These dynamic responses in net NO_3^-, NH_4^+, and NO_2^- fluxes following the additions of NO_3^-, compared to minimal net fluxes of NO_3^- and NO_2^- before the additions (Tables 2, 3, 4), indicate that N transformations were far more dynamic at the respective sites than would be recognized from observing only net fluxes of the ions without additions.

The increases in positive NO_2^- fluxes accounted for 11 to 58% of the increases in NO_3^- removal rates in August (mean 23%, SE 5.5, $n = 8$) and about 5 to 13% (mean 8%, SE 1.7, $n = 4$) of the increases in November. This pattern was not as clear in January, when most NO_3^- removal, NO_2^- accumulation, and DNRA rates (see below) were low.

N_2 production rates, NH_4^+ fluxes, and potential DNRA rates

Net N_2 production rates (in μmol N m^{-2} h^{-1}) at the four stations ranged from near zero at Rabbit in August to about 350 at Duck in January (Fig. 2). Overall, N_2 production rates were highest in January (range 95 to 357; Fig. 3), except for Rankin Key, where January and November rates were comparable. Mean net N_2 effluxes (5 to 87) before $^{15}NO_3^-$ additions indicated low denitrification rates in August (Fig. 2). Increases in N_2 fluxes after $^{15}NO_3^-$ additions were observed in most experiments (Fig. 3 vs. 2), but differences were not consistent among stations, and rates occasionally decreased after additions.

In contrast to results for N_2 flux, $^{15}NO_3^-$ additions in August had a pronounced effect on NH_4^+ release rates (μmol N m^{-2} h^{-1}) (Table 2). Potential DNRA rates, measured as $^{15}NH_4^+$ accumulation after $^{15}NO_3^-$ addition, ranged from about 40 to 170 in August (Fig. 4) and correlated significantly with net NH_4^+ release rates before ($r^2 = 0.53$, $p < 0.05$, $n = 8$) and after ($r^2 = 0.81$, $p < 0.01$, $n = 8$) $^{15}NO_3^-$ additions to the same cores. Potential DNRA rates were lowest in January, when they ranged from 11 to 82 (Fig. 4), and did not correlate significantly with net NH_4^+ release rates before or after $^{15}NO_3^-$ additions. With the exception of Murray, DNRA rates in November were comparable to or higher (Rabbit) than August results. At Murray, DNRA rates were more than twice as high in August (110–134) as in January or November (53, 54; Fig. 4). The most pronounced seasonal difference for DNRA occurred at Rabbit, where $^{15}NH_4^+$ accumulation rates were about 40 in August and 10 in January, but increased in November to about 240, the highest $^{15}NH_4^+$ accumulation rate observed in our study. Note that Florida Bay was experiencing a large picocyanobacteria bloom in November, which was intense at the Rabbit site and least significant at Murray (McCarthy et al. 2009).

Relative importance of DNRA vs. denitrification to the fate of NO_3^-

The ratios of net N_2 flux to measured total NH_4^+ flux (DNF:TAF) examined before and after addition of $^{15}NO_3^-$ (Table 5) provide one approach to gain

Table 5 The mean ratios of net denitrification rates (DNF) to total NH_4^+ fluxes (TAF) before and after (August and January) $^{15}NO_3^-$ additions or controls (without) vs. $^{15}NO_3^-$ additions (November)

	Rabbit	Murray	Rankin	Duck
August				
DNF:TAF (before)	0.08	0.30	0.14	0.28
DNF:TAF (after)	1.60	0.08	0.10	0.12
January				
DNF:TAF (before)	1.5	1.7	6.0	1.2
DNF:TAF (after)	1.5	0.94	1.6	0.61
November				
DNF:TAF (without)	0.28	0.24	1.40	0.19
DNF:TAF (with)	0.08	0.03	−0.11*	0.15

August and January (before) results represent the average results from Experiments 1 and 2, whereas January (after) results are only from Experiment 1 (because $^{15}NO_3^-$ was not added in Experiment 2)

* Negative value caused by N_2 uptake (apparent net N_2 fixation)

Table 6 Atom % ^{15}N in the total regenerated NH_4^+ and N_2, respectively, after $^{15}NO_3^-$ additions

	Rabbit	Murray	Rankin	Duck
August				
Atom % ^{15} N of TAF	23	25	26	30
Atom % ^{15}N of N_2	37	44	0	26
January				
Atom % ^{15}N of TAF	15	16	28	15
Atom % ^{15}N of N_2	1	5	0	17
November				
Atom % ^{15}N of TAF	32	29	33	34
Atom % ^{15}N of N_2	68	93	88	17

Note that the $^{15}NO_3^-$ was added after incubating cores to obtain background rates in August and January experiments, whereas it was added at the beginning of incubations in November experiments

insights about the relative importance of denitrification vs. DNRA in determining the fate of NO_3^-. A high ratio provides evidence for denitrification as the dominant process, whereas a low ratio favors the importance of DNRA and/or high NH_4^+ remineralization rates. In August, DNF:TAF before isotope addition was 0.3 or less at all of the sites, indicating that NH_4^+ dominated N_2 as the major reduced N compound produced. However, in January, the opposite pattern was observed; net N_2 fluxes before addition ranged from being similar to about six times higher than NH_4^+ fluxes. November results were intermediate between August and January results, with DNF:TAF ranging from 0.2 to 1.4 before isotope addition. Except for Rabbit results in August and January, DNF:TAF decreased consistently in response to the addition of $^{15}NO_3^-$ at the different stations and sampling times, indicating that $^{15}NO_3^-$ added to the water was used more effectively for DNRA than for denitrification at most sites.

In August, ^{15}N accounted for 23–30 % of total NH_4^+ flux and 26–44 % of total N_2 flux after isotope addition, except for Rankin, where none of the measured N_2 was derived from $^{15}NO_3^-$ (Table 6). In January, ^{15}N accounted for 15–16 % of NH_4^+ flux after isotope addition, except for Rankin, where it was 28%, but ^{15}N accounted for only 1, 5, 0, and 17 % of N_2 at the respective sites (Table 6). In November, ^{15}N accounted for 29–34% of the total NH_4^+ flux and 17–93% of the total N_2 flux. In contrast to the August and January results of almost no ^{15}N being incorporated into N_2 at Rankin, ^{15}N accounted for 88% of the November N_2 produced at Rankin after isotope addition. In most cases, $^{15}NO_3^-$ was incorporated into a comparable or higher fraction of N_2 flux than NH_4^+ flux in August and November (Table 6). The opposite trend was observed at 3 out of 4 sites in January, when denitrification was a more dominant process regulating the fate of in situ NO_3^-.

Potential anammox rates in January and November

The absence of $^{29}N_2$ production after addition of $^{15}NH_4^+$ in January (data not shown) and November (Fig. 5) suggest that anammox was not an important process affecting N dynamics at the sediment—water interface in Florida Bay. In contrast, some $^{29}N_2$ production was observed in the presence of added $^{15}NO_3^-$, presumably from denitrification (Fig. 5).

Relationship of N_2 flux and potential DNRA rates to total sediment O_2 demand

Denitrification (net N_2 fluxes before isotope additions and in controls) and DNRA rates were compared to SOD rates to examine relationships of these processes to consumption of O_2 in the same cores. Significant relationships were observed in August and January (Figs. 6, 7) but not in November, when the results

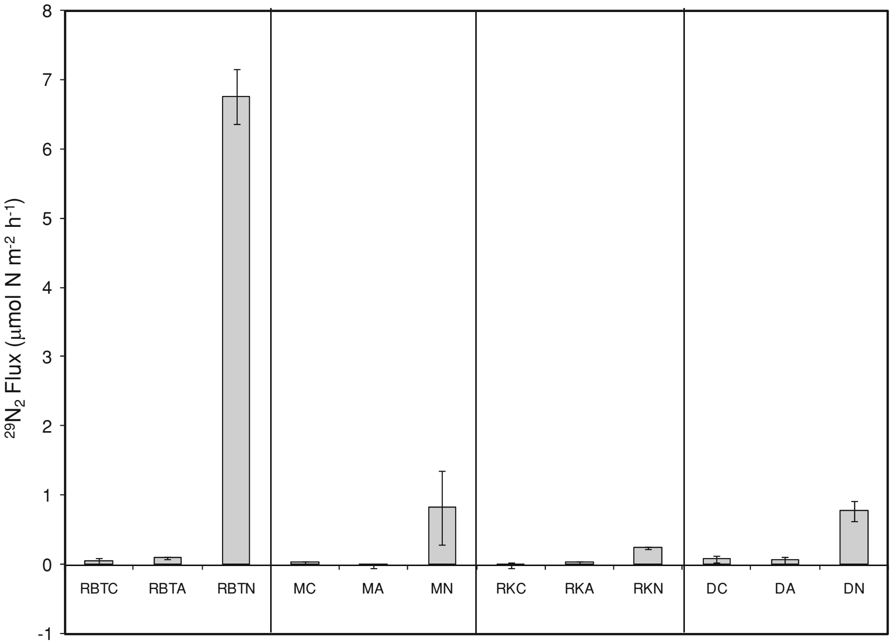

Fig. 5 Production rates of $^{29}N_2$ for control cores (C) and those treated with $^{15}NH_4^+$ (A) or $^{15}NO_3^-$ (N), respectively, at the four stations in November 2006. It is assumed that $^{29}N_2$ production after the addition of $^{15}NH_4^+$ may reflect potential anammox, whereas that formed after $^{15}NO_3^-$ addition could be formed from either anammox or traditional denitrification. Error bars reflect one standard error. RBT = Rabbit; M = Murray; RK = Rankin; D = Duck

may have been affected by the picocyanobacteria bloom. Net N_2 flux in August and January related significantly ($p < 0.1$) to SOD before $^{15}NO_3^-$ additions within each sampling period, but denitrification rates were much higher in January than in August, despite a lower SOD (Fig. 6). Note that our SOD results may be conservative because of permeability of the Teflon tubing to O_2 diffusion, but this effect has not been a decipherable except in hypoxic waters, which create a strong oxygen gradient across the walls of the tubing (unpublished data from hypoxic zones of Lake Erie and the northern Gulf of Mexico).

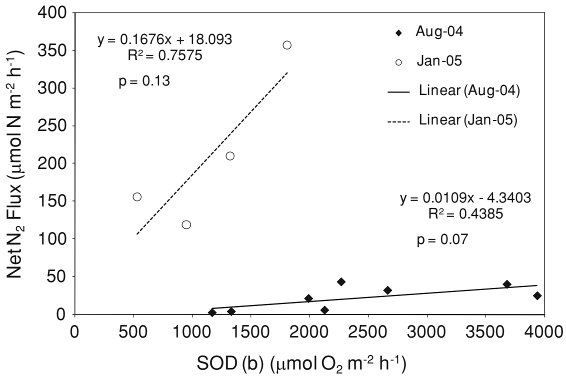

Fig. 6 Relationship of net N_2 production to sediment oxygen demand (SOD) for cores from the four Florida Bay sites in August 2004 and January 2005

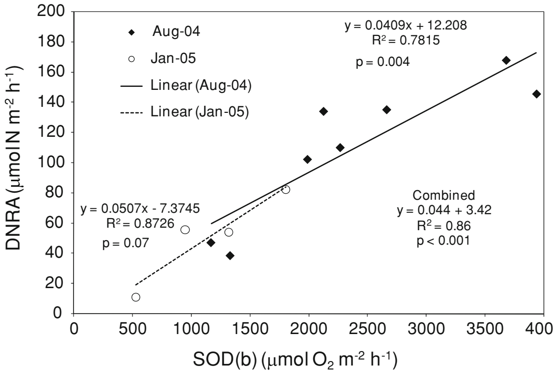

Fig. 7 Relationship of potential DNRA to sediment oxygen demand (SOD) for cores from the four Florida Bay sites in August 2004 and January 2005

A significant relationship was observed between potential DNRA rates and SOD, not only within but also among the two sampling periods of August and January (Fig. 7), but not in November. In November, an exceptionally high DNRA:SOD ratio was observed at Rabbit Key, where the oxygen-producing picocyanobacteria bloom was pronounced. Note that the August and January comparison on Fig. 7 was based on the SOD's measured after the $^{15}NO_3^-$ additions to correspond with the periods used for DNRA measurements, but the relationship was significant whether the SOD values were taken before or after the additions.

Discussion

Overall, the data collected at the four representative Florida Bay sites in August, January, and November support our original hypothesis, *"DNRA is a significant process retaining bioavailable fixed N in Florida Bay by producing* NH_4^+ *as a dissimilatory end product of* NO_3^- *reduction,"* and allow the three related questions to be addressed below:

(*1*) *What is the relative importance of DNRA and DNRN versus* N_2 *production (denitrification or anammox) in controlling the fate of N?* Distinguishing the role of DNRA (plus DNRN) vs. denitrification to the fate of NO_3^- is not trivial because nitrification-coupled DNRA cannot be differentiated from heterotrophic production of NH_4^+ (or NO_2^-) resulting from bacterial decomposition or animal excretion. Therefore, comparing the measured NH_4^+, NO_3^-, and NO_2^- fluxes to N_2 fluxes provided insights about biogeochemical transformations and exchanges of N between the sediments and water, but did not distinguish the roles of DNRA, versus mineralization or excretion, as NH_4^+ production processes. Assessing potential DNRA rates by simply measuring $^{15}NH_4^+$ accumulation rates after addition of $^{15}NO_3^-$ to the inflowing water was conservative because it did not include the DNRA coupled directly to in situ nitrification. Also, the exchange reactions between $^{15}NH_4^+$ and $^{14}NH_4^+$ near the DNRA reaction sites at the redox transition zone (Morse and Morin 2005) may have prevented some $^{15}NH_4^+$ from diffusing to the sediment surface within the time scale of the experiments and caused DNRA to be underestimated (Gardner et al. 2006). However, combining the two approaches provide important insights about the fate of N in Florida Bay. For example, a significant correlation of measured DNRA with net NH_4^+ flux before (and after) $^{15}NO_3^-$ additions in August supports the concept that DNRA is an important source of NH_4^+ accounting for the high fluxes of NH_4^+ across the sediment–water interface.

Our data provides strong evidence that DNRA affects N dynamics and nutrient ratios seasonally in Florida Bay. In August, measured N_2 fluxes were small (4 to 15 %) relative to total NH_4^+ fluxes at the four sites. In contrast, during January, N_2 fluxes were up to six times as high as NH_4^+ fluxes before NO_3^- addition (Table 5). In November, N_2 production rates were lower than total NH_4^+ fluxes at all sites except Rankin, where the two rates were comparable. Likewise, N_2 fluxes were lower than $^{15}NH_4^+$ fluxes at all four sites in August and November (Figs. 2, 4) but comparable or higher in January (Figs. 3, 5). The significant correlation between DNRA (Fig. 4) and measured NH_4^+ flux, before ($p < 0.05$) or after ($p < 0.01$) $^{15}NO_3^-$ addition (Table 2), in August is consistent with the hypothesis that DNRA was a dominant source of NH_4^+ from the sediments during summer. Cumulatively, these data suggest that DNRA was the dominant NO_3^- reduction process and provided an important "link" for bioavailable N during August and November, but denitrification, represented by N_2 flux, was more dominant as a "sink" in January.

The low N_2 production rates observed in August may have been caused by inhibition of denitrification due to increased sulfide production or lack of NO_3^- substrate. The latter cause seems unlikely because DNRA requires the same NO_3^- substrate as denitrification. DNRA is favored over denitrification by the presence of sulfate reducing bacteria because sulfide inhibits the final step of denitrification but enhances DNRA (Brunet and Garcia-Gil 1996). Porewater sulfide was not measured in August, but a noticeable sulfide odor was observed in August surface sediments within a short period after samples were collected. The available carbon to NO_3^- ratio may affect the partitioning between denitrification and DNRA (Tiedje 1988). DNRA is favored over denitrification by high labile organic carbon availability and low NO_3^- concentrations (Megonigal et al. 2004 and references therein). For example, DNRA is often dominant under fish cages in coastal regions with fish farms (Christensen et al. 2000). Consistent with the organic carbon concept, highest DNRA rates were observed at the Rabbit site in November (Fig. 4) during the picocyanobacteria bloom, which may have supplied labile organic matter to the DNRA microbes. Fall blooms, common in this region (Phlips et al. 1999; Glibert et al. 2004), may be related to wind-driven movements of water from the north-central region into the south-central region of the basin (Phlips et al. 1999).

Temperature and salinity may also be important factors regulating the fate of NO_3^-. In agreement with the high DNRA rates observed in August, high temperatures favored DNRA in temperate estuarine sediments (King and Nedwell 1984). A diagenetic model (Kelly-Gerreyn et al. 2001) predicted that denitrification is the favored pathway at temperatures between 14 and 17°C, but DNRA is favored in warmer or cooler temperatures outside of that range. DNRA rates may be enhanced by elevated salinities (Megonigal et al. 2004; Gardner et al. 2006), as occurred at our Florida Bay sites.

Examination of NO_2^- concentration and production patterns provides further insight about the importance of DNRA and DNRN to N dynamics at the different sites. The accumulation of NO_2^- in association with DNRA is common (Kelso et al. 1999). Our field measurements indicate that NO_2^- was present in overlying water (Table 1). This consistent result was initially surprising because NO_2^- is not normally expected to be a stable end-product in aquatic systems (Maier et al. 2000). However, previous investigators have noted increasing NO_2^- concentrations within the bay (Boyer et al. 1999). It is an intermediate product in several N transformation processes (Kelso et al. 1999).

Our experimental results suggest that the NO_2^- observed may reflect active DNRA and DNRN. Nitrite accumulation rates increased markedly after $^{15}NO_3^-$ addition in August and November, and on average accounted for ca. 23 and 8% of the total removal of NO_3^- during those respective months (from data in Table 2). Although lower, NO_2^- accumulation rates (Tables 2, 4) were sometimes comparable to the DNRA rates estimated as $^{15}NH_4^+$ fluxes (Fig. 4), suggesting that (1) NO_2^- may have been an important product of dissimilatory NO_3^- reduction, and (2) uptake processes for $^{15}NO_2^-$, formed during DNRA, were not as rapid as NO_2^- production rates after $^{15}NO_3^-$ was added. The accumulation of NO_2^- may relate to sulfide dynamics. Ammonium was the main dissimilatory product during sulfide oxidation in Lake Vilar, Spain, but NO_2^- became more important as sulfide was depleted (Brunet and Garcia-Gil 1996). In our experiments, the pattern of high potential NO_2^- production was not observed in January, when denitrification rates were high, relative to DNRA, and a smaller fraction of N_2 and NH_4^+ was derived from the added $^{15}NO_3^-$ at 3 of 4 sites (Table 6). The above results indicate that NO_2^- may be an important product of dissimilatory NO_3^- reduction in Florida Bay, particularly in August, when DNRA processes dominated in Florida Bay. These results also may help explain why NO_2^- is often measurable in Florida Bay waters (Boyer et al. 1999).

(2) *Is NO_3^- supplied in the water reduced as efficiently to N_2 or NH_4^+ as that formed by in situ nitrification?* Observing the atom % ^{15}N of N_2 and NH_4^+ after addition of high concentrations of $^{15}NO_3^-$ to inflow waters provides insights about potential NO_3^- sources for denitrification and DNRA, respectively, and helps determine the potential influence of NO_3^- from the water column on measured N-dynamics. Differentiating between in situ coupled nitrification-denitrification from denitrification fueled by water column NO_3^- (e.g., Nielsen 1992) is important because the former process can be attributed mostly to internal N regeneration and conversion processes, whereas the latter process may simulate the fate of NO_3^- originating from tributaries or other outside sources especially during episodic events. Our experiments thus offer insights about the potential effects of the NO_3^- source for the respective dissimilatory reduction processes at the different sites, characterized by varying nutrient ratios and degrees of eutrophication. Increased atom % ^{15}N of N_2 and NH_4^+ in the outflow waters after $^{15}NO_3^-$ addition suggests effective use of NO_3^- supplied from overlying water, whereas an increased fraction of $^{28}N_2$ or $^{14}NH_4^+$ implies that the reduced NO_3^- was more coupled to nitrification of natural-abundance NH_4^+ within the ecosystem, presumably near the redox transition zone. Interestingly, most of the NO_3^- substrate for both dissimilatory reactions was coupled to in situ nitrification rather than to NO_3^- in overlying water, even after excess $^{15}NO_3^-$ additions (Table 6). Except for denitrification results at three stations in November, the percentage of ^{15}N was smaller than ^{14}N in both the N_2 and NH_4^+ end products, even though inflow water contained high levels of added $^{15}NO_3^-$.

Results from the eutrophic Rankin Key are noteworthy in that no ^{15}N-label was observed in the N_2 produced in August and January. In contrast, at the other three sites, 26 to 44 % of the total N_2 flux was derived from $^{15}NO_3^-$ in August. Seventeen to 93% of total N_2 was derived from the added $^{15}NO_3^-$ at the four sites in November, versus only 1 to 17 % in January, when background N_2 fluxes were high relative to DNRA rates (Table 5). These data suggest that sediment NO_3^- supply rates may have been more limiting to denitrification in August and November than in January. The opposite pattern was observed for DNRA. $^{15}NH_4^+$ accounted for 26, 28 and 33 atom % of the total NH_4^+ flux at the Rankin site in August, January, and November, respectively (Table 5). Thus, conditions at this eutrophic site were conducive to reducing added NO_3^- via DNRA but not via denitrification in August and January. High DNRA to denitrification ratios for NO_3^- derived from water passing over enriched sediments could help explain the general observation that denitrification efficiency often decreases with increased inputs of tributary NO_3^- (Mulholland et al. 2008; Seitzinger 2008), at least for the Florida Bay system.

DNRA may be less O_2-sensitive than denitrification, because DNRA bacteria can be aerobic, facultatively anaerobic, or obligately anaerobic (Megonigal et al. 2004). Thus, in Rankin sediments with high SOD, NO_3^- from the overlying water may have been subjected to conditions favorable to DNRA. The presence of sulfide, which is inhibitory to denitrification but conducive to DNRA, does not offer a complete explanation for these results because coupled denitrification was apparent at the Rankin site, but almost none of the produced N_2 was derived from the added ^{15}N in August and January.

(*3*) *How do these key N-transformation processes relate to season, sampling site, and/or SOD rates?* As is clear from the above discussion, seasonal differences in N-dynamics were pronounced. DNRA (with associated DNRN) was the dominant dissimilatory NO_3^- reduction process at all four sites in August when SOD rates were high and denitrification rates were low. In January, N_2 production was greater than NH_4^+ production, including DNRA, but the magnitudes of the two processes were comparable. In general, November results were intermediate, with both DNRA and denitrification as important dissimilatory NO_3^- reduction processes.

Both DNRA and denitrification rates correlated with SOD within the same sampling period in August and January. In contrast, DNRA rates were related directly to SOD rates over a wide range, for data collected in August and January, but the relationship did not extend through the November data. Part of the reason that this relationship did not hold in November may have been due to oxygen production by the picocyanobacteria, which would have reduced the measured net SOD, as the incubations were conducted under dampened light, to simulate natural light conditions. However, the highest $^{15}NH_4^+$ accumulation rates also occurred in November at Rabbit Key during the algal bloom. This result may suggest the importance of "high-quality" fresh organic matter to the DNRA process. Cyanobacteria blooms were associated with high levels of dissolved organic N and P in central Florida Bay (Glibert et al. 2004).

An alternate explanation could be that the increased $^{15}NH_4^+$-accumulation may have resulted from rapid conversion of the $^{15}NO_3^-$ to $^{15}NH_4^+$ by assimilatory recycling processes associated with the algal bloom (assimilatory nitrate reduction to ammonium: ANRA). This possibility appears unlikely at the sediment–water interface because ANRA would have been inhibited by NH_4^+ (Tiedje 1988), which was released consistently at high rates from the sediments before and after $^{15}NO_3^-$ additions at our Florida Bay stations (Tables 2, 3, 4). If ANRA were significant in the over-flowing water, added $^{15}NO_3^-$ should have been converted to $^{15}NH_4^+$ within the inflow reservoirs used for the sediment core incubations. However, NH_4^+ accumulation combined with NO_3^- removal was not observed in inflow reservoirs. In fact, total NO_3^- concentration increased steadily, and there were no significant increases in the atom % ^{15}N for NH_4^+ in inflow reservoirs during the November incubations (data not shown). Therefore, we believe that DNRA provides a more likely explanation than ANRA for accumulation of $^{15}NH_4^+$ in our Florida Bay experiments.

Conceptual summary of N dynamics in Florida Bay

Continuous-flow experiments indicated that N-dynamics at the sediment–water interface are active in Florida Bay and support a preliminary conceptual framework of N-dynamics in shallow subtropical, and probably tropical, coastal ecosystems with high salinities. Our data suggest that DNRA and associated DNRN are important mechanisms for retaining bioavailable fixed N as NH_4^+ or NO_2^- in Florida Bay, especially in summer. Potential DNRA and associated total NH_4^+ flux were higher or comparable to denitrification rates in August and November, but denitrification rates were higher than DNRA rates in January. We did not measure the ^{15}N content of NO_2^-, but the patterns and amounts of NO_2^- accumulation in response to $^{15}NO_3^-$ additions suggest that NO_2^- formation by DNRN was associated more closely with DNRA than with denitrification. High rates of dissimilatory NH_4^+ and NO_2^- production, relative to denitrification (or anammox), serves as a "link" to keep reactive N in the system and help explain why bioavailable N:P and [NH_4^+ plus NO_2^-]:NO_3^- ratios are high in some regions of Florida Bay. Similar results may be expected in other sub-tropical and tropical regions. High DNRA rates, observed in warm regions under hypersaline conditions (An and Gardner 2002; Megonigal et al. 2004; Gardner et al. 2006), and supported here by Florida Bay results, help resolve the controversy about the importance of high salinities (Zieman et al. 1999) versus increased nutrient loadings (Lapointe and Clark 1992) as important factors in seagrass die-offs and associated ecosystem changes in Florida Bay. Texas coastal (Gardner et al. 2006) and Florida Bay data (this paper) suggest that these two factors are interactive, along with temperature, redox conditions, and organic carbon availability. High salinities and temperature and organic carbon availability may exacerbate the effects of nutrient inputs by maintaining bioavailable N in the system as NH_4^+ or NO_2^- via DNRA or DNRN rather than having it removed via denitrification or anammox. At eutrophic sites, this effect may be manifested more strongly for NO_3^- in water passing over the sediments (as from tributary inputs) than for that produced from in situ nitrification. The results from this study demonstrate a close relationship between N- and O_2-dynamics, which are both affected by eutrophication.

Acknowledgments We thank Lisa Tipsword, the boat captains, and other Keys Marine Laboratory personnel for laboratory and vessel support, Jim Cotner, Frank Jochem, Peter Lavrentyev, Zhang Lu, Ted Stets, and Clayton Williams for discussions and field assistance, Associate Editor Jan Mulder and two unidentified reviewers for constructive suggestions, and Jiqing Liu for reading the final manuscript. This research was sponsored by the NOAA Coastal Ocean Program National Center for Coastal Ocean Science, Grant # NA04NOS4780017.

References

An S, Gardner WS (2002) Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen "link", versus denitrification as a "sink" in a shallow estuary (Laguna Madre/Baffin Bay, Texas). Mar Ecol Prog Ser 237:41–50. doi:10.3354/meps237041

An S, Gardner WS, Kana T (2001) Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. Appl Environ Microbiol 67:1171–1178. doi:10.1128/AEM.67.3.1171-1178.2001

Bianchi TS, Pennock JR, Twilley RR (eds) (1999) Biogeochemistry of Gulf of Mexico Estuaries. John Wiley Inc., New York, p 428

Boyer JN, Fourqurean JW, Jones RD (1997) Spatial characterization of water quality in Florida Bay and Whitewater Bay by multivariate analysis: zones of similar influence (ZSI). Estuaries 20:743–758. doi:10.2307/1352248

Boyer JN, Fourqurean JW, Jones RD (1999) Seasonal and long-term trends in the water quality of Florida Bay (1989–1997). Estuaries 22:417–430. doi:10.2307/1353208

Brunet RC, Garcia-Gil LJ (1996) Sulfide-induced dissimilitory nitrate reduction to ammonia in anaerobic freshwater sediments. FEMS Microbiol Ecol 21:131–138. doi:10.1111/j.1574-6941.1996.tb00340.x

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Rev Ecol Soc Am 5(2):89–96. www.frontiersinecology.org

Christensen PL, Rysgaard S, Sloth NP, Dalsgaard T, Schwaerter S (2000) Sediment mineralization, nutrient fluxes, denitrification and dissimilatgory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. Aquat Microb Ecol 21:73–84. doi:10.3354/ame021073

Cornwell JC, Kemp WM, Kana TM (1999) Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. Aquat Ecol 33:41–54. doi:10.1023/A:1009921414151

Cotner JB, Sada RH, Bootsma H, Johengen T, Cavaletto JF, Gardner WS (2000) Nutrient limitation of heterotrophic bacteria in Florida Bay. Estuaries 23:611–620. doi:10.2307/1352888

Dalsgaard T, Thamdrup B (2002) Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. Appl Environ Microbiol 68:3802–3808. doi:10.1128/AEM.68.8.3802-3808.2002

Fourqurean JW, Robblee MB (1999) Florida Bay: a history of recent ecological changes. Estuaries 22:345–357. doi:10.2307/1353203

Fourqurean JW, Jones RD, Zieman JC (1993) Processes influencing water column nutrient characteristics and phosphorus limitation of phytoplankton biomass in Florida Bay, FL, USA: inferences from spatial distributions. Estuar Coast Shelf Sci 36:295–314. doi:10.1006/ecss.1993.1018

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martineli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–897. doi:10.1126/science.1136674

Gardner WS, Bootsma HA, Evans C, St. John PA (1995) Improved chromatographic analysis of ^{15}N:^{14}N ratios in ammonium or nitrate for isotope dilution experiments. Mar Chem 48:271–282. doi:10.1016/0304-4203(94)00060-Q

Gardner WS, McCarthy MJ, An S, Sobolev D, Sell KS, Brock D (2006) Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnol Oceanogr 51:558–568 (Special issue on eutrophication)

Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar Ecol Prog Ser 280:73–83. doi:10.3354/meps280073

Horner-Devine MC, Martiny AC (2008) News about nitrogen. Science 320:757–758. doi:10.1126/science.1147012

Joye SB, Hollibaugh JT (1995) Influence of sulfide inhibition on nitrification on nitrogen regeneration in sediments. Science 270:623–625. doi:10.1126/science.270.5236.623

Kana TM, Darkangelo C, Hunt MD, Oldham JB, Bennett GE, Cornwell JC (1994) Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Anal Chem 66:4166–4170. doi:10.1021/ac00095a009

Kelly-Gerreyn BA, Trimmer M, Hydes DJ (2001) A diagenetic model discriminating denitrification and dissimilatory nitrate reduction to ammonium in a temperate estuarine sediment. Mar Ecol Prog Ser 220:33–46. doi:10.3354/meps220033

Kelso BHL, Smith RV, Laughlin RJ (1999) Effects of carbon substrates on nitrite accumulation in freshwater sediments. Appl Environ Microbiol 65:61–66

King D, Nedwell DB (1984) Changes in the bacteria nitrate-reducing community of an anaerobic saltmarsh sediment in response to seasonal selection by temperature. J Gen Microbiol 130:2935–2941

Koch MS, Erskine JM (2001) Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: interactions with light, salinity and temperature. J Exp Mar Biol Ecol 266:81–95. doi:10.1016/S0022-0981(01)00339-2

Koike I, Hattori A (1978) Denitrification and ammonium formation in anaerobic coastal sediments. Appl Environ Microbiol 35:278–282

Kuypers MM, Sliekers AO, Lavik G, Schmid M, Jorgensen BB, Kuenen JG, Sinninghe Damsté JS, Strous M, Jetten MS (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422:608–611. doi:10.1038/nature01472

Lapointe BE, Barile PJ (2004) Comment on J.C. Zieman, J.W. Fourqurean, and T.A. Frankovich. 1999. Seagrass dieoff in Florida Bay: long-term trends in abundance and growth of turtle grass, *Thalassia testudinum*. Estuaries 22:460–470. Estuaries and Coasts 27:157–176. doi:10.1007/BF02803569

Lapointe BE, Clark MW (1992) Nutrient inputs from the watershed and coastal eutrophication in the Florida Keys. Estuaries 15:465–476. doi:10.2307/1352391

Lavrentyev PJ, Bootsma HA, Johengen TH, Cavaletto JF, Gardner WS (1998) Microbial plankton response to resource limitation: insights from the cummunity structure and seston stoichiometry in Florida Bay, USA. Mar Ecol Prog Ser 165:45–57. doi:10.3354/meps165045

Lavrentyev PJ, Gardner WS, Yang L (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment–water interface. Aquat Microb Ecol 21:187–194. doi:10.3354/ame021187

Maier RM, Pepper IL, Gerba CP (2000) Environmental microbiology. Academic Press, San Diego

McCarthy MJ, Gardner WS, Lavrentyev PL, Jochem FJ, Williams CJ (2009) Water column nitrogen cycling and microbial plankton in Florida Bay. Contrib Mar Sci (in press)

McCarthy MJ, Lavrentyev PL, Yang L, Zhang L, Chen Y, Qin B, Gardner WS (2007) Nitrogen dynamics relative to microbial food web structure in a subtropical, shallow, well-mixed, eutrophic lake (Taihu Lake, China). Hydrobiologia 581:195–207. doi:10.1007/s10750-006-0496-2 (special issue on Lake Taihu)

Megonigal JP, Hines ME, Visscher PT (2004) Anaerobic metabolism: Linkages to trace gasses and aerobic processes. In: Schlesinger WH (ed) Biogeochemistry. Elsevier-Pergamon, Oxford, pp 317–424

Morse JW, Morin J (2005) Ammonium interaction with coastal marine sediments: influence of redox conditions on K*. Mar Chem 95:107–112. doi:10.1016/j.marchem.2004.08.008

Mulholland PJ et al (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–205. doi:10.1038/nature06686

Nielsen LP (1992) Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol Ecol 86:35–362. doi:10.1111/j.1574-6968.1992.tb04828.x

Phlips EJ, Badylak S (1996) Spatial variability in phytoplankton standing crop and composition in a shallow inner-shelf lagoon, Florida Bay, Florida. Bull Mar Sci 58:203–216

Phlips EJ, Lynch TC, Badylak S (1995) Chlorophyll a, tripton, color, and light availability in a shallow tropical inner-shelf lagoon, Florida Bay, USA. Mar Ecol Prog Ser 127:223–234. doi:10.3354/meps127223

Phlips EJ, Badylak S, Lynch TC (1999) Blooms of the picoplanktonic cyanobacterium *Synechococcus* in Florida Bay, a subtropical inner-shelf lagoon. Limnol Oceanogr 44:1166–1175

Robblee MB, Barber TR, Carlson PR, Durako MJ, Fourqurean JW, Muehlstein LK, Porter D, Yarbro LA, Zieman RT, Zieman JC (1991) Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay. Mar Ecol Prog Ser 71:297–299. doi:10.3354/meps071297

Rysgaard S, Risgaard-Petersen N, Sloth NP (1996) Nitrification, denitrification, and nitrate ammonification in sediments of two coastal lagoons in southern France. Hydrobiologia 329:133–141. doi:10.1007/BF00034553

Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr 33:702–724

Seitzinger SP (2008) Nitrogen cycle out of reach. Nature 425:162–163. doi:10.1038/452162a

Sørensen J (1978) Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. Appl Environ Microbiol 35:301–305

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. New York, John Wiley, pp 179–243

Zieman JC, Fourqurean JW, Frankovich TA (1999) Seagrass die-off in Florida Bay: Long-term trends in abundance and growth of turtle grass, *Thalassia testudinum*. Estuaries 22:460–470. doi:10.2307/1353211