Journal of Coastal Research	00	0	000–000	West Palm Beach, Florida	Month 0000

Benthic Nitrogen Fixation in an Eutrophic Estuary Affected by Groundwater Discharge

Alexandra M.F. Rao* **and Matthew A. Charette**

Department of Marine Chemistry and Geochemistry
Woods Hole Oceanographic Institution
Woods Hole, MA 02543, U.S.A.

www.cerf-jcr.org

ABSTRACT

RAO, A.M.F. and CHARETTE, M.A., 2012. Benthic nitrogen fixation in an eutrophic estuary affected by groundwater discharge. *Journal of Coastal Research,* 00(0), 000–000. West Palm Beach (Florida), ISSN 0749-0208.

Anthropogenic nutrient loading of coastal aquifers has resulted in the discharge of nutrient-enriched groundwater to the coastal zone, with sweeping repercussions on aquatic community structure and ecosystem function. Groundwater-derived nutrient loading is known to initiate coastal eutrophication, hypoxia, and harmful algal blooms. However, most land-derived inputs of nitrogen to the coastal ocean are thought to be removed in heterotrophic estuarine and coastal sediments, which have traditionally been considered net fixed nitrogen sinks, reducing nutrient export to the oceans. Here, we provide evidence of net N_2 fixation in heterotrophic estuarine sands affected by submarine groundwater discharge based on $N_2:Ar$ measurements in benthic flux chamber incubations. Below the surface sediments, we observed efficient removal of fixed N. Together, these results suggest that net N_2 fixation in sandy estuarine sediments is promoted by N limitation because of efficient fixed N removal in subsurface aquifer sediments and in muddy sediments elsewhere in this estuarine system. Our observations suggest that estuarine sediments in some anthropogenically affected ecosystems may not represent a net fixed N sink, limiting the potential attenuation of increased N loads and permitting increased N export to the open ocean.

ADDITIONAL INDEX WORDS: *Nitrogen fixation, denitrification, nitrogen cycling, marine sediments, Waquoit Bay, groundwater.*

INTRODUCTION

Human activities in the past century have resulted in a tremendous increase in nutrient inputs to estuaries worldwide, promoting increases in harmful algal bloom frequency, phytoplankton biomass and production, hypoxic and anoxic bottom waters, eutrophication, turbidity, nutrient export to the oceans, and alterations in estuarine nutrient cycling and ecosystem dynamics (Fisher *et al.,* 2006; Glibert *et al.,* 2008; Howarth and Marino 2006). Submarine groundwater discharge (SGD) is a major conduit for natural and anthropogenic nutrient inputs to the coastal zone (Andersen *et al.,* 2007; Capone and Bautista 1985; Paytan *et al.,* 2006). The discharge of nutrient-enriched groundwater, resulting from chronic anthropogenic nutrient loading of coastal aquifers, has sweeping repercussions on aquatic community structure and function (*e.g.,* Bowen and Valiela 2001; Herrera-Silvera and Morales-Ojeda 2009; Hu, Muller-Karger, and Swarzenski, 2006).

The effect of groundwater nutrient loads on coastal waters is controlled in part by biogeochemical transformations in sediments in three aquifer regions, illustrated in Figure 1: within the freshwater plume, the freshwater–seawater mixing zone (the subterranean estuary [STE]), and at the seepage face (Charette, Sholkovitz, and Hansel, 2005; Kroeger and Charette 2008; Slomp and Van Cappellen 2004). Although previous studies have focused on the fresh groundwater zone, biogeochemical transformations in the STE and at the seepage face are not well understood.

Although oceanographers have long argued that phosphorus (P) is the ultimate master variable controlling productivity in aquatic ecosystems on geologic timescales and both mesocosm and whole-ecosystem studies have corroborated this view in lakes (*e.g.,* Schindler *et al.,* 2008), there is a growing body of evidence suggesting that productivity and eutrophication in most temperate, coastal ecosystems are limited by nitrogen (N). Planktonic N fixation, which alleviates short-term N limitation in many freshwater systems, is generally absent at higher salinities. This dichotomy may be explained by a complex set of controls on N fixation, including physical factors, such as light availability and water column stability; chemical interferences, such as sulfate inhibition of molybdenum uptake in cyanobacteria; and ecological controls, such as zooplankton grazing pressure (Howarth and Marino 2006; Marino *et al.,* 2006; Paerl 2009; Vitousek et al., 2002,). Although nitrogen limitation is known to favor microbial N_2 fixation and benthic N_2 fixation has been previously reported in various coastal systems (*e.g.,* Jones 1982; O'Neil and Capone 1989), estuarine sediments have traditionally been regarded as nitrogen sinks, where microbial fixed N removal attenuates terrestrial and anthropogenic

---

DOI: 10.2112/JCOASTRES-D-11-00057.1 received 1 April 2011; accepted in revision 20 September 2011.

* Present address: Vrije Universiteit Brussel (VUB), Analytical and Environmental Chemistry, Earth System Sciences, Pleinlaan 2, 1050 Brussel, Belgium, (e-mail: a.rao@nioo.knaw.nl).

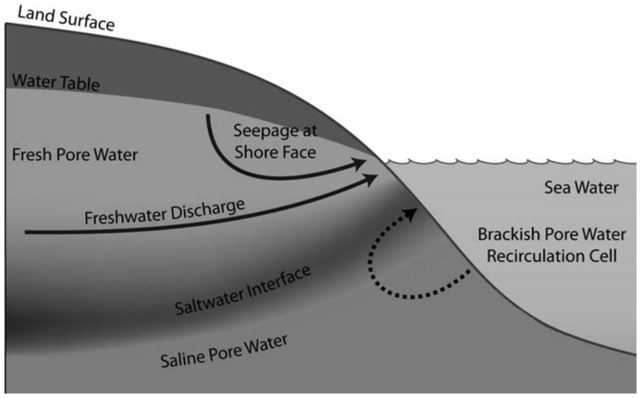

Figure 1. A schematic diagram of a subterranean estuary.

nitrogen inputs to the coastal ocean (Seitzinger 1988). However, recent studies have suggested that some coastal sediments may be in balance with respect to microbial nitrogen sources and sinks (*e.g.,* Gardner *et al.,* 2006), whereas others may even display net nitrogen fixation (Fulweiler *et al.,* 2007).

In this study, we examined nitrogen fluxes and transformations at Waquoit Bay (Cape Cod, Massachusetts) in permeable sediments in the shallow subterranean estuary and at the groundwater seepage face using a push–pull experiment and benthic flux chamber incubations. We show that efficient fixed N removal in the shallow STE at this site can completely consume groundwater N inputs to this zone, but seepage face sediments may still represent a net fixed N source to this estuarine system because of net benthic N_2 fixation.

MATERIALS AND METHODS

Study Site

Waquoit Bay is a semienclosed estuary in Cape Cod, Massachusetts, with an average depth of approximately 1 m. Rainfall infiltration and rapid groundwater flow are facilitated by the permeable soils on Cape Cod. The bay is flushed with water from Vineyard Sound, Massachusetts, to the south. Several studies have documented the significance of SGD in Waquoit Bay (*e.g.,* Charette, Sholkovitz, and Hansel, 2005; Michael, Mulligan, and Harvey, 2005), which represents 80% of freshwater inputs to the bay. The unconfined surface aquifer that contributes most of the freshwater discharge at the study site in the head of Waquoit Bay (Figure 2) consists of relatively homogeneous, permeable sediment, resulting in a well-confined STE (Charette, Sholkovitz, and Hansel, 2005; Mulligan and Charette 2006). Surface sediments at this site consist of 95% sand and <5% silt and clay, with 0.05% to 0.075% organic carbon (Charette, Sholkovitz, and Hansel, 2005;). Hydraulic conductivity in surface aquifer sediments ranges from 32 to 150 m d^{-1} (Mulligan and Charette 2006).

Removal of dissolved inorganic nitrogen (DIN) in aquifer sediments in Waquoit Bay, Massachusetts, has previously been suggested from measurements of ϕ^{1}[illegible] enrichment in NO_3^- and NH_4^+ in both shallow and deep salinity transition zones (Kroeger and Charette 2008). Denitrification has also been measured previously in fine-grained sediments throughout this estuarine system (Lamontagne *et al.,* 2002). Despite DIN removal, higher anthropogenic N loads in Waquoit Bay in the 20th century have resulted in increased eutrophication, dissolved N, phytoplankton and macroalgal biomass, and loss of eelgrass (Bowen and Valiela 2001).

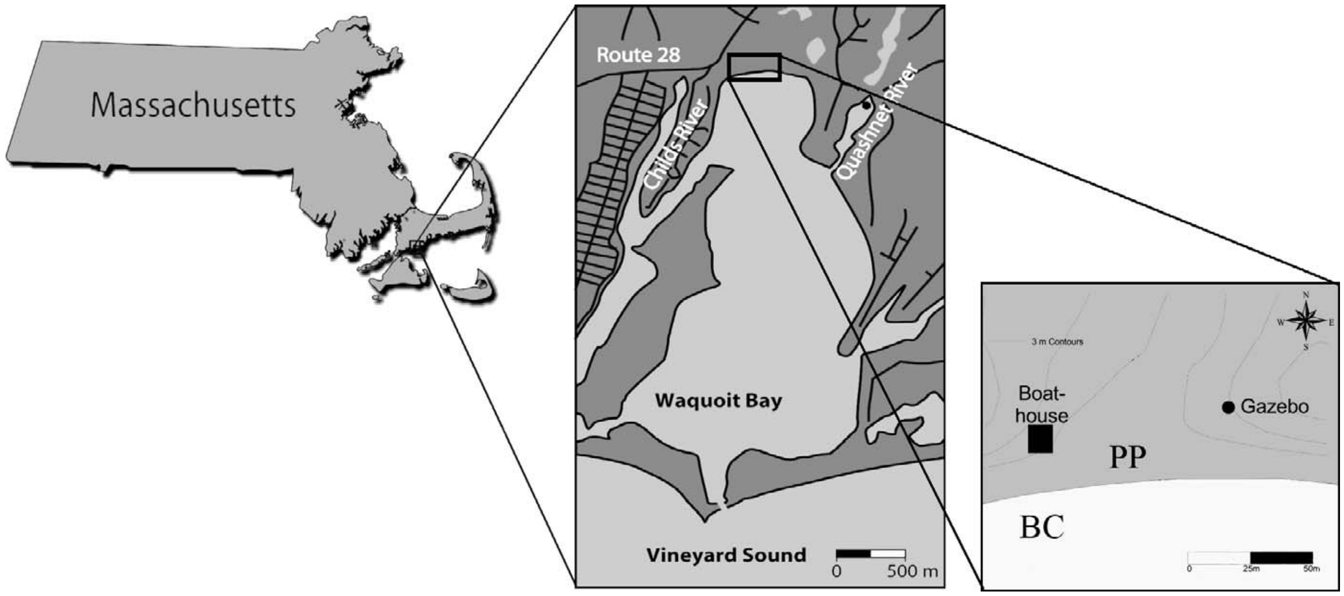

Figure 2. Study site at the head of Waquoit Bay, Massachusetts. A push–pull experiment (PP) was conducted at the intertidal zone in the valley between the two bluffs in June 2008, and benthic flux chamber incubations (BC) were conducted bayward of the low-tide line, in front of the boathouse.

Sample Collection and Analytical Methods

To investigate NO_3^- reduction *in situ* in the shallow STE within the nearshore groundwater recirculation zone in June 2008, we used an AMS Retract-A-Tip piezometer (Charette and Allen 2006) inserted at 45 cm depth to extract groundwater for NO_3^- and Br^- spike addition, reinject the spiked groundwater, and sample the plume periodically after reinjection following the push–pull technique (Addy *et al.,* 2005). To minimize atmospheric contamination and heating of the extracted groundwater, approximately 40 L of groundwater was pumped through Viton tubing into a preevacuated gas sampling bag (Calibrated Instruments, Inc., Hawthorne, New York), kept on ice, and prefilled with unlabelled NO_3^- and conservative tracer (Br^-) spikes to final concentrations of approximately 200 μM and 40 mM, respectively. Following mixing and reinjection, the groundwater plume was resampled hourly at the same position during 4 hours. Ancillary groundwater properties at the site of the push–pull experiment were measured with a YSI (Yellow Springs, Ohio) 600R sonde with temperature, salinity, dissolved oxygen, and pH/oxidation-reduction potential (ORP) sensors.

Light and dark benthic flux chambers were used to examine carbon and nitrogen transformations at the groundwater seepage face. Flux chambers, which are acrylic cylinders with an inner diameter of 20 cm, were implanted manually into the sediment, enclosing approximately 20 cm of sediment with 10–15 cm of overlying water. Advective porewater exchange was maintained within the enclosed sediments by acrylic rods, which stirred the overlying water continuously at 40 rpm. Laboratory experiments have shown that this stirring rate corresponds to a pressure gradient of 2.9 Pa between the center and the rim of the flux chamber, which is in the range of *in situ* pressure gradients resulting from unidirectional flow over bottom topography (Janssen *et al.,* 2005). Groundwater discharge into the overlying water enclosed by the chamber was permitted in alternate incubations (May 16 and 30; June 25 and 27) by pressure equalization through a capillary tube open to the surrounding bay water. Temperature and salinity fluctuations in the chamber overlying water were monitored continuously using a YSI multiprobe sonde screwed into the flux chamber lids at the start of each chamber incubation. After flux chamber lids were secured, a NaBr tracer was added at the start of all incubations to a final concentration of 3–5 mM in chamber overlying water. The dilution of this tracer was used to quantify the overlying water volume and the rate of porewater exchange in the flux chambers. A water sample of 80–100 mL was collected hourly for solute analyses as described below. We report results from eight pairs of flux chamber incubations in May and June 2008 at the head of Waquoit Bay just below the low-tide line in about 1 m water depth. Net sediment-water solute fluxes were determined from time-series chamber water samples. In three deployments (May 29 and 30 and June 27), an $^{15}NO_3^-$ spike was added at the start of incubations to a final concentration of approximately 200 μM in the chamber overlying water for the determination of denitrification rates by the isotope pairing technique. Although isotope pairing results are not shown here, NO_3^- concentration time series reflect this spike addition.

Calculations of gross benthic respiration and photosynthesis rates assumed a 1 : 1 $O_2 : C$ ratio. Daily benthic photosynthesis rates were calculated by assuming that benthic photosynthesis proceeds between 1 hour after sunrise and 1 hour before sunset, based on sunrise–sunset tables obtained from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneYear.php). Calculated daily benthic respiration rates assumed that benthic respiration rates measured in the dark flux chambers proceeded continuously.

Rates of porewater advection in the flux chamber incubations were estimated by subtracting the rate of solute transport by diffusion, obtained using a finite difference model of diffusive solute exchange (Rao and Jahnke 2004), from the total exchange rates calculated from linear fits of Br^- time-series data. Model-estimated diffusive exchange represents <5% of the measured solute exchange rates. In principle, chamber solute fluxes should be corrected for dilution with bottom water at each sample collection time, but because the loss of Br^- tracer during deployments was generally low (mean 21%) and because this error is likely offset by uncertainties due to changing interfacial concentrations during chamber deployment (Bender *et al.,* 1989), this correction has been ignored (Jahnke and Jahnke 2000).

Water samples for nutrient analyses (nitrate, ammonium, dissolved organic nitrogen) were collected in plastic syringes, filtered (0.2 μm Sterivex, Millipore, Billerica, Massachusetts) into 30 mL, acid-cleaned scintillation vials, and frozen until analysis by standard methods on a Lachat Instruments (Loveland, Colorado) QuickChem 8000 flow injection analyzer. Filtered and refrigerated samples were analyzed colorimetrically for bromide (Presley 1971). Water samples from flux chamber and push–pull experiments for dissolved N_2 and O_2 analyses were collected in glass syringes and filled into slender 15 mL gas-tight Exetainer vials (Labco, H[illegible]ycombe, U.K.) with screw caps, preserved with 200 mL of 50% $ZnCl_2$, and stored under water until analysis by membrane inlet mass spectrometry (MIMS; Balzers Prisma QME 200 with Channeltron/Faraday detection, Pfeiffer Vacuum, Asslar, Germany) at the University of Texas Marine Science Institute (An, Gardner, and Kana, 2001; Kana *et al.,* 1994). Sample vials were filled from the bottom with a 5 mL overflow volume, preventing significant exchange with atmospheric N_2. Samples were stored refrigerated to avoid bubble formation and were analyzed within 6 weeks of collection. Dissolved gas concentrations were determined from MIMS analyses using the solubility constants of Weiss (1970). Average measurement precision for dissolved gases (O_2, Ar, $^{28}N_2$: coefficient of variation 0.03%) was calculated from replicate ($n = 13$) measurements of two standards at 0 ppt salinity maintained in 21°C and 30°C temperature baths.

It has been suggested that MIMS $N_2 : Ar$ analyses may suffer from a potential artifact associated with scavenging of N_2 by O^+ ions, formed by the fragmentation of O_2 in the ion source of the mass spectrometer, producing NO^+ (m/z 30; Eyre *et al.,* 2002). Tests conducted with a copper-reduction column inline with the mass spectrometer used in this study indicated that O_2 concentrations have little effect on measured $N_2 : Ar$ (S. An, unpublished results).

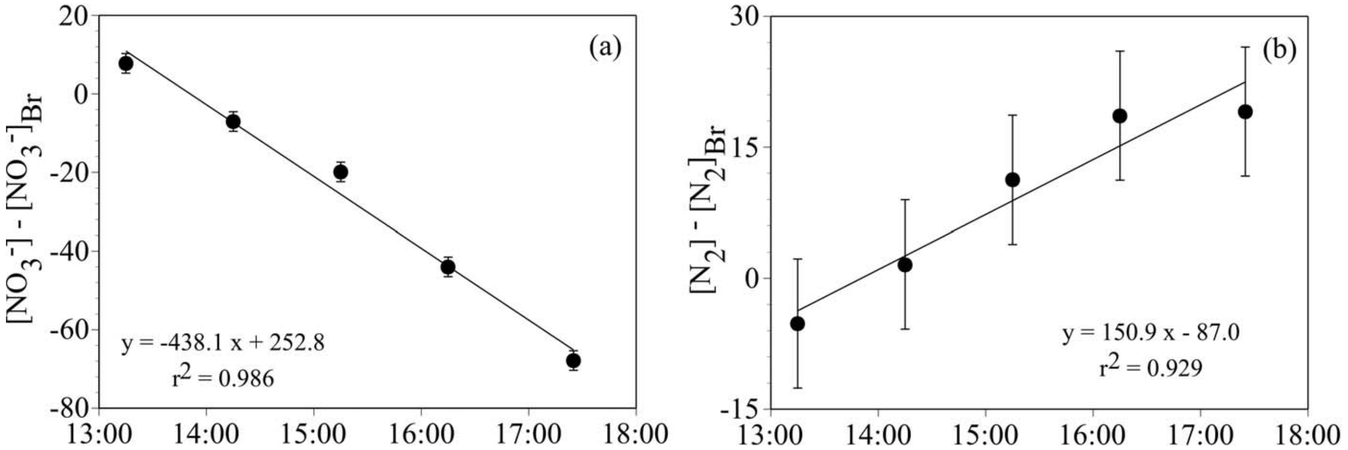

Figure 3. Temporal changes in (a) NO_3^-, and (b) N_2 concentrations (μmol L^{-1}) in the spiked groundwater plume during the June 2008 push–pull experiment. Concentrations are expressed as the difference between measured values and the concentrations expected from conservative mixing of the spiked groundwater plume, based on the dilution of Br^- tracer. Error bars (±1 standard deviation [SD], $n = 3$) represent propagated average analytical uncertainty. Regressions shown were used to calculate NO_3^- consumption and N_2 production rates.

RESULTS

Groundwater at the push–pull experiment site was brackish (21–22 ppt) and suboxic (15–20 μM O_2), with low total dissolved nitrogen: $NO_3^- < 1$ μM, $NH_4^+ = 10-40$ μM, and dissolved organic N = 5–20 μM. The NO_3^- added to the shallow STE during this experiment was removed at a rate $\left(-R_{NO_3-N}\right)$ of 7.3 mmol N m^{-3} sediment h^{-1}, and N_2 was produced at 5.0 mmol N m^{-3} sediment h^{-1} (Figure 3).

Calculated advective exchange rates during flux chamber incubations (93 ± 44 and 227 ± 101 L m^{-2} d^{-1} in May and June 2008) agree with porewater exchange rates measured previously with similar chambers in field and laboratory experiments (Glud, Forster, and Huettel, 1996; Janssen *et al.,* 2005).

Temperature and salinity in benthic flux chamber overlying water varied by up to 2°C and 2 ppt, respectively (Table 1). Measured temperature and salinity variations in chamber overlying water were used to calculate flux chamber dissolved gas concentrations from dissolved gas ratios measured in the flux chamber overlying water samples by MIMS (An, Gardner, and Kana, 2001).

Net O_2 consumption indicated benthic respiration in all dark chamber incubations, whereas benthic photosynthesis resulted in lower net O_2 consumption or net O_2 production in light chamber incubations (Figure 4). Calculated benthic respiration rates (4.35 ± 1.98 and 6.31 ± 1.44 mmol C m^{-2} h^{-1} in May and June) exceeded gross photosynthesis (2.64 ± 0.63 and 3.45 ± 1.60 mmol C m^{-2} h^{-1} in May and June).

A reversal in benthic net N_2 flux was observed in dark chambers, from releases indicating net denitrification rates up to 0.38 mmol N m^{-2} h^{-1} in mid-May to uptake, indicating net nitrogen fixation rates up to 0.77 mmol N m^{-2} h^{-1} in late May and June (Figure 4). Note that only dark chamber N_2 fluxes are presented here.

In unamended incubations, benthic fluxes of NH_4^+ (Figure 5) are higher and more variable than NO_3^- fluxes (Figure 6). Increased NO_3^- influx was observed in $^{15}NO_3^-$–amended flux chamber incubations relative to unamended incubations (Table 2, Figure 6). Measured NH_4^+ fluxes did not vary consistently between unamended and amended incubations but increased in magnitude and variability after mid-May (Figure 5). Measured areal DIN fluxes in unamended incuba-

Table 1. *Summary of ranges in measured flux chamber temperature and salinity.*

	Temperature (°C)		Salinity (ppt)	
	Light	Dark	Light	Dark
15 May 2008	14.87–15.29	14.73–15.01	28.45–28.86	26.06–26.24
16 May 2008	14.39–14.71	14.39–14.51	31.05–31.1	27.85–27.92
29 May 2008	19.72–21.17	19.07–20.28	25.47–26.45	23.03–23.58
30 May 2008	21.07–22.55	20.9–21.7	28.92–29.41	25.71–26.0
24 June 2008	24.58–26.41	24.33–25.79	25.73–25.99	23.34–23.73
25 June 2008	25.57–27.01	25.39–26.05	30.21–30.58	27.65–27.96
26 June 2008	24.17–24.64	24.03–24.45	23.3–24.48	21.48–22.08
27 June 2008	24.35–25.62	24.17–25.09	26.1–26.48	23.79–24.43

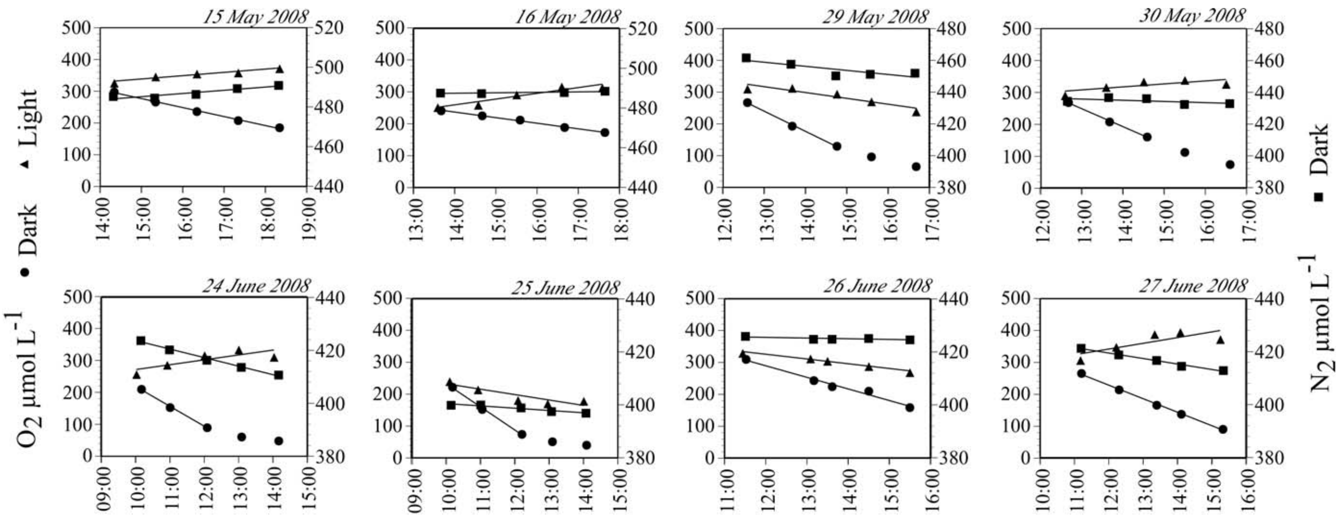

Figure 4. Time series measurements of overlying water O_2 and N_2 concentrations in light (O_2 only) and dark (O_2 and N_2) benthic flux chamber incubations. Error bars representing average analytical uncertainty (±1 SD, $n = 3$) are smaller than the symbols in each case. Solid lines represent the slopes used to calculate benthic fluxes. In dark chamber incubations where benthic O_2 fluxes were clearly reduced because of low chamber water O_2 concentrations (May 29, May 30, June 24, and June 25), only the first three measurements were used to calculate benthic O_2 fluxes.

tions (Table 2) are comparable to fluxes reported in other estuarine systems (Gardner *et al.,* 2006; Giblin *et al.,* 2010).

DISCUSSION

The push–pull experiment revealed rapid $^{15}NO_3^-$ reduction to N_2 in suboxic aquifer sediments at the head of Waquoit Bay, at a rate of 5.0 mmol N m^{-3} sediment h^{-1} (Figure 3). The difference between measured $^{15}NO_3^-$ removal and N_2 production rates $\left(R_{NO_3-N}+R_{N_2-N} = -2.3 \text{ mmol N m}^{-3} \text{ sediment h}^{-1}\right)$ may be due to dissimilatory nitrate reduction to ammonium, although only a low rate of net NH_4^+ consumption (0.41 mmol N m^{-3} sediment h^{-1}) was observed during the push–pull experiment. Alternatively, some N_2 loss may have resulted from

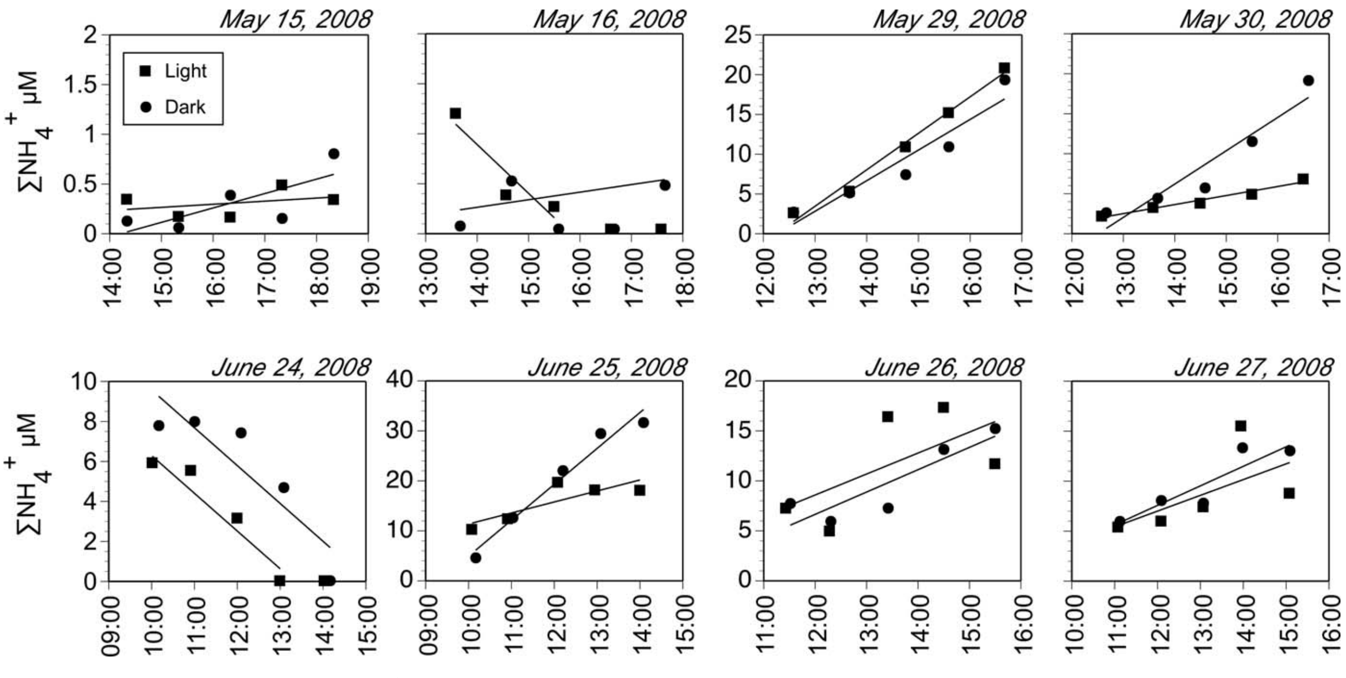

Figure 5. Time series measurements of NH_4^+ concentrations in light and dark benthic flux chamber incubations. Solid lines represent the slopes used to calculate benthic NH_4^+ fluxes. In the light chamber on May 16, NH_4^+ flux was clearly reduced because of low chamber water NH_4^+ concentrations, so only the first three measurements were used to calculate benthic fluxes. Error bars representing average analytical uncertainty (±1 SD, $n = 3$) are smaller than the symbols.

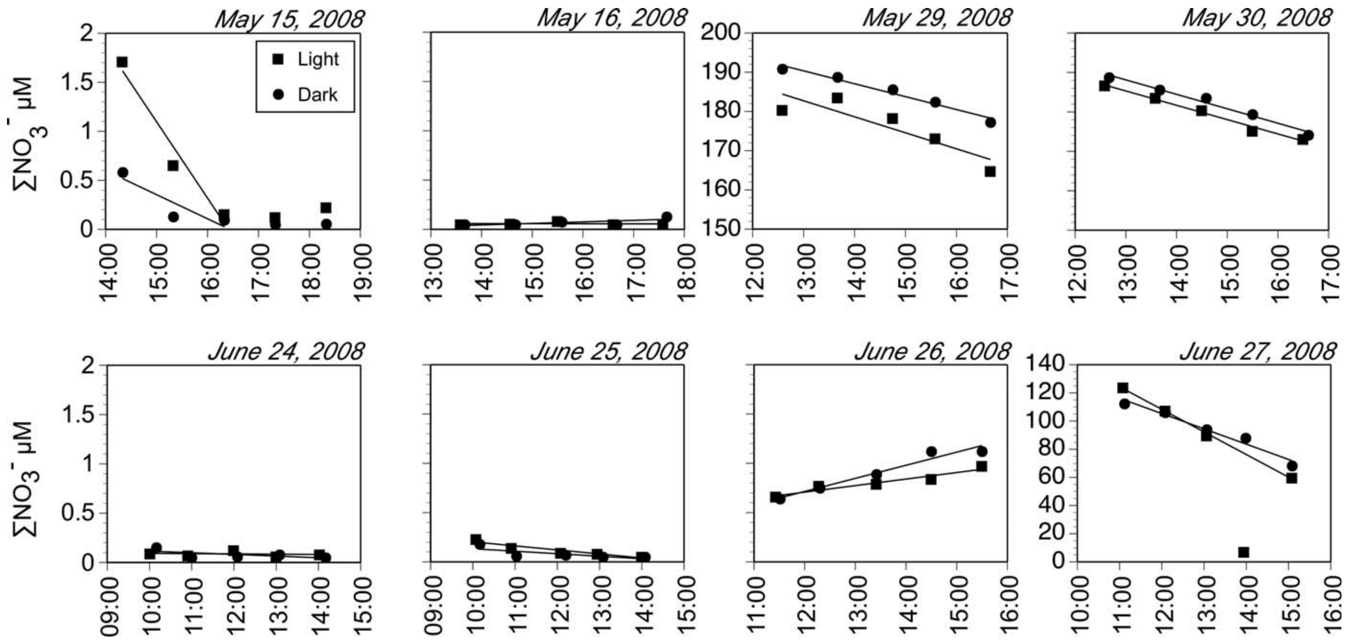

Figure 6. Time series measurements of overlying water NO_3^- ($NO_3^- + NO_2^-$) concentrations in light and dark benthic flux chamber incubations. Solid lines represent the slopes used to calculate benthic NO_3^- fluxes. In chamber incubations where benthic fluxes were clearly reduced because of low chamber water NO_3^- concentrations (May 15), only the first three measurements were used to calculate benthic fluxes. Note the higher concentration scales required for the May 29, May 30, and June 27 data. The $^{15}NO_3^-$ additions raised chamber water NO_3^- concentrations in these incubations. Error bars representing average analytical uncertainty (±1 SD, $n = 3$) are smaller than the symbols.

pressure variations during sampling. Previous observations suggest that nitrate plumes in the underlying freshwater aquifer mix into the shallow salinity transition zone (Kroeger and Charette 2008), where they are likely depleted by the high N removal rates observed in this experiment. It is difficult to calculate the total N loss rate in aquifer sediments before discharge based on our observations; therefore, these results must be cautiously interpreted. However, the N removal rates observed in this study are sufficient to completely deplete even the highest NO_3^- concentrations previously observed here in the shallow STE (Kroeger and Charette 2008), assuming that groundwater travels at approximately 1 m d^{-1} within a 1-m-deep nearshore groundwater recirculation zone extending 3 m from the high-tide mark (Michael, Mulligan, and Harvey, 2005).

Benthic flux chamber filtration rates most likely overestimate the rate of advective exchange between porewater and the overlying chamber water because measured Br^- tracer fluxes also reflect the loss of tracer from groundwater discharging in the area enclosed by each flux chamber. However, no consistent differences in Br^- tracer fluxes were observed in replicate chambers with and without groundwater discharge, probably because of spatiotemporal variability in groundwater discharge rates (Mulligan and Charette 2006). Although this variability prohibits the as[illegible]ent of groundwater discharge rates during the fluxchamber experiments conducted here, previous studies have observed and quantified high discharge rates at this site (Michael, Mulligan, and Harvey, 2005; Mulligan and Charette 2006).

Benthic photosynthesis and respiration rates derived from benthic chamber O_2 fluxes demonstrate that sands at the study site are net heterotrophic, but they nevertheless support high rates of benthic photosynthesis and contribute significantly to total ecosystem production, which reaches 19 mmol O_2 m^{-2} h^{-1} in summer (D'Avanzo, Kremer, and Wainright, 1996). Linear regression showed that water temperature explains much of the observed variability in benthic respiration ($p = 0.01$), but not in benthic photosynthesis ($p = 0.25$), which is more likely controlled by light availability (*e.g.,* Berg and Huettel 2008;

Table 2. *Meas[illegible] benthic chamber fluxes (light/dark). For $^{28}N_2$ fluxes, only dark chamber results are presented. Boldface columns indicate* $^{15}NO_3^-$ –amended *incubations.*

mmol m^{-2} h^{-1}	May 15 (14.9°C)	May 16 (14.5°C)	May 29 (20.2°C)	May 30 (21.6°C)	June 24 (25.4°C)	June 25 (26.1°C)	June 26 (24.4°C)	June 27 (24.9°C)
O_2 flux	1.381/−3.280	2.113/−2.131	**−2.071/−6.396**	**1.030/−5.586**	2.101/−7.395	−2.110/−7.660	−2.107/−4.737	**2.008/−5.443**
NH_4^+ NH_4+ flux	0.004/0.017	−0.057/0.009	**0.516/0.380**	**0.125/0.415**	−0.278/−0.225	0.275/0.761	0.297/0.292	**0.177/0.252**
NO_3^- NO_3− flux	−0.103/−0.028	0.000/0.002	**−0.460/−0.325**	**−0.402/−0.365**	0.000/−0.002	−0.005/−0.003	0.009/0.017	**−1.803/−1.401**
$^{28}N_2$ flux	0.380	0.058	**−0.410**	**−0.034**	−0.768	−0.173	0.005	**−0.410**

Jahnke *et al.,* 2008). Benthic respiration rates in permeable, sandy sediments observed in this study were remarkably similar to those reported previously in muddy sediments elsewhere in Waquoit Bay (Lamontagne *et al.,* 2002).

Kroeger and Charette (2008) estimated benthic DIN inputs at the head of Waquoit Bay in the summer of 2003 from terrestrial and marine-source groundwater, of 371 and 44 mmol N (m shoreline)$^{-1}$ d^{-1}, respectively, with terrestrial groundwater inputs mostly (82%) in the form of NO_3^- and marine-source groundwater inputs in the form of NH_4^+. These estimates were derived from the nutrient composition of fresh and saline groundwater in the subterranean estuary and previous estimates of fresh and saline groundwater discharge at this site from Darcy's Law, seepage meter, and radiochemical tracer measurements. It is difficult to compare these estimates of groundwater-derived nutrient inputs with nutrient fluxes estimated here from *in situ* flux chamber incubations, in part, because of differences in the spatial scales considered with these different approaches. Although Kroeger and Charette (2008) integrated indirect calculations of coastal groundwater nutrient inputs over large spatial scales, benthic flux chamber estimates provide a direct measure of benthic nutrient fluxes on smaller spatial scales, which incorporate the effect of microbial processes and nitrogen cycling in surface sediments. With these caveats in mind, we can make this comparison based on DIN flux at the seepage face (mmol [m shoreline]$^{-1}$ d^{-1}). Assuming the seepage face extends to 40 m from shore (Michael, Mulligan, and Harvey, 2005), the DIN fluxes measured in benthic flux chamber incubations in this study (Figures 5 and 6) range from −71 to 1 mmol NO_3^- (m shoreline)$^{-1}$ d^{-1} and −32 to 9 mmol NH_4^+ (m shoreline)$^{-1}$ d^{-1} in May, and from −4 to 12 mmol NO_3^- (m shoreline)$^{-1}$ d^{-1} and −248 to 436 mmol NH_4^+ (m shoreline)$^{-1}$ d^{-1} in June.

Two important results of this analysis warrant highlighting. First, in comparison with earlier groundwater DIN flux estimates by Kroeger and Charette (2008), the large variability in benthic nutrient flux estimates from *in situ* flux chamber incubations illustrates the importance of spatial and temporal heterogeneity in nitrogen transformations in surface sediments. These results suggest that benthic DIN fluxes at the head of Waquoit Bay are dependent on environmental conditions (*e.g.,* temperature, wave height, turbidity, benthic light levels, organic matter supply), which control microbial nitrogen cycling pathways at the seepage face. Second, earlier groundwater DIN flux estimates suggested that benthic DIN inputs are largely in the form of NO_3^- (Kroeger and Charette 2008), while benthic flux chamber measurements suggest that benthic NH_4^+ fluxes are larger and more variable. Again, this difference most likely reflects the difference in spatial scales considered by each study. Although Kroeger and Charette (2008) focused on DIN speciation to 10 m depth in the subterranean estuary at the head of Waquoit Bay, Massachusetts, the benthic flux chamber measurements discussed here provide a direct measure of nutrient fluxes at the seepage face and their response to both microbial nitrogen transformations in surface sediments and groundwater inputs from below.

The imbalance between total $^{15}NO_3^-$ consumed and ^{15}N released by $^{29}N_2$ and $^{30}N_2$ production in amended incubations (data not shown), suggests that these incubations were not at steady state with respect to NO_3^- following spike addition. However, at least two features in the amended flux chamber data sets are worth considering. Measured NO_3^- influx in amended incubations was greater in light than in dark conditions (Table 2). This difference is likely due to uptake by benthic microalgae and macroalgae. Furthermore, NO_3^- influx in amended light and dark incubations was greater at higher temperatures in June than it was in May, consistent with the temperature-dependence of metabolic rates.

Benthic N_2 fluxes indicative of net denitrification in mid May are in the range of those reported in fine-grained sediments elsewhere in this estuarine system (Lamontagne *et al.,* 2002). In contrast to the low net denitrification rates observed in mid May, the measured net benthic nitrogen fixation rates observed at this site in late May and June are, to our knowledge, among the highest ever observed in marine sediment. Many bacteria in marine sediments are capable of carrying out N_2 fixation, including heterocystous and nonheterocystous aerobic cyanobacteria; anaerobes (sulfate reducers, methanotrophs); and facultative anaerobes (Berman-Frank *et al.,* 2003; Coyer *et al.,* 1996; Jones 1982; Reddy *et al.,* 1993). Diurnal fluctuations in N_2 fixation are determined by the local microbial community because some organisms fix N only in the light, others do so only in darkness, and yet others carry out N_2 fixation independent of ambient light intensity (Jones 1982, O'Neil and Capone 1989). Further molecular studies are needed to identify the organisms that mediate N transformations in these sediments and to determine the dependence of N_2 fixation on environmental parameters, such as light intensity.

The discovery of significant benthic nitrogen fixation in this eutrophic estuary plagued by historic biomass increases (Bowen and Valiela 2001) is consistent with early reports that nitrogen fixation rates in shallow, coarse-grained marine sediments are higher in eutrophic than oligotrophic sites and are generally stimulated by organic matter additions (O'Neil and Capone 1989 and references therein). It has been suggested that organic substrate availability may enhance heterotrophic nitrogen fixation in marine sediments by promoting the consumption of O_2 in bulk sediments or in sediment microenvironments, thereby protecting the enzyme nitrogenase from O_2 inactivation (O'Neil and Capone 1989 and references therein). Although our results suggest that benthic nitrogen fixation is enhanced in eutrophic systems with increased organic matter inputs, Fulweiler *et al.* (2007) have also demonstrated enhanced net benthic nitrogen fixation due to reduced denitrification rates in an estuarine system undergoing oligotrophication (Fulweiler *et al.,* 2007). Furthermore, it is well known that increased organic substrate (and DIN) availability associated with eutrophication also enhances denitrification rates (Seitzinger 1988). The effect of these and other factors on the interplay between benthic nitrogen fixation and denitrification and on net benthic N_2 flux warrants further study.

CONCLUSIONS

These flux chamber measurements indicate that sandy sediments at the seepage face at the head of Waquoit Bay represent a net fixed N source to the bay due to net benthic N_2 fixation in late spring and summer. The rapid N transformations observed in the shallow STE and at the seepage face in this study illustrate the importance of sediment transformation rate and benthic flux measurements in studies of SGD fluxes of nutrients to the coastal ocean. Furthermore, the high N_2 fixation rates measured here are comparable only to rates observed in Narragansett Bay, Rhode Island, where it was recently shown that oligotrophication and declining organic matter deposition may cause muddy coastal sediments to switch from their traditional role as net sinks to net sources of fixed N (Fulweiler *et al.,* 2007). We suggest that net benthic N_2 fixation in sandy sediments at Waquoit Bay, an eutrophic estuary, may be promoted by low N levels and N limitation due to efficient fixed N removal in muddy sediments elsewhere in this estuarine system and in aquifer sediments just below the seepage face, as observed in the June 2008 push–pull experiment, and at depth (Kroeger and Charette 2008; Lamontagne *et al.,* 2002).

To test the potential significance of this benthic N source on an ecosystem scale, we assume that the net benthic N_2 fixation rates observed in this study can be applied to sandy sediments around the upper half of the bay circumference (Cambareri and Eichner 1998). Using the minimum measured net N_2 fixation rate gives an input of 9650 mol N d^{-1}. During the 1.5-month study period, this input is equivalent to 25% of the annual atmospheric, wastewater, and fertilizer N inputs combined (Bowen and Valiela 2001), and it balanced 35% of the annual net benthic N sinks in fine-grained Waquoit Bay sediments (Lamontagne *et al.,* 2002). We are not yet in a position to extrapolate these results to annual estimates because N transformation rates in Waquoit Bay are subject to high seasonal variability (Lamontagne *et al.,* 2002). More-detailed studies will be required to examine the spatiotemporal variability of benthic fixed N sources and sinks, and to determine the extent to which benthic N_2 fixation in surface sands may balance annual benthic N sinks in this estuarine system. If the temperature dependence of benthic N_2 fixation mirrors the temperature and organic substrate dependence of denitrification, then both processes may peak in summer, and net benthic N_2 fixation in sandy sediments may decline in fall and winter as both processes weaken and as increased dissolved N availability in bay water reduces N limitation.

Evidence of net N_2 fixation and less efficient fixed N removal in coastal sediments underlines the importance of efforts to reduce nutrient loading in coastal catchments from a variety of different sources, including wastewater and stormwater systems; failing septic systems; industrial, commercial, and construction runoff; and overapplication of mineral fertilizers and organic wastes in lawns and agricultural soils. Studies have shown that in some watersheds, groundwater transport of nutrients may match, or at times even exceed, surface water transport (Bachman *et al.,* 1998; Simonds *et al.,* 2008). These results confirm that improved management strategies, including further actions to regulate groundwater nutrient loading in coastal watersheds with permeable soils, such as in Cape Cod, are necessary to achieve improved water quality in coastal ecosystems.

ACKNOWLEDGMENTS

We thank P. Henderson, M. McCarthy, and W. Gardner for analytical assistance; M. Gonneea for fieldwork assistance; and the Waquoit Bay National Estuarine Research Reserve (NERR) for access to their waters and facilities. A.M.F.R. and M.A.C. were supported by the U.S. National Science Foundation (NSF OCE-0524994).

LITERATURE CITED

Addy, K.; Gold, A.; Nowicki, B., McKenna, J., Stolt, M., and Groffman, P., 2005. Denitrification capacity in a subterranean estuary below a Rhode Island fringing salt marsh. *Estuaries,* 28, 896–908.

An, S.; Gardner, W.S., and Kana, T., 2001. Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. *Applied and Environmental Microbiology,* 67, 1171–1178.

Andersen, M.S.; Baron, L.; Gudbjerg, J.; Gregersen, J.; Chapellier, D.; Jakobsen, R., and Postma, D., 2007. Discharge of nitrate-containing groundwater into a coastal marine environment. *Journal of Hydrology,* 336, 98–114.

Bachman, L.J.; Lindsey, B.D.; Brakebill, J.W., and Powars, D.S., 1998. Groundwater Discharge and Base-Flow Nitrate Loads of Nontidal Streams, and Their Relation to a Hydrogeomorphic Classification of the Chesapeake Bay Watershed, Middle Atlantic Coast. Baltimore, Maryland: U.S. Geological Survey Water-Resources Investigations Report 98-4059, 72p.

Bender, M.; Jahnke, R.; Weiss, R.; Martin, W.; Heggie, D.; Orchardo, J., and Sowers, T., 1989. Organic carbon oxidation and benthic nitrogen and silica dynamics in San Clemente Basin, a continental borderland site. *Geochimica et Cosmochimica Acta,* 53, 685–697.

Berg, P. and Huettel, M., 2008. Monitoring the seafloor using the noninvasive eddy correlation technique: Integrated benthic exchange dynamics. *Oceanography,* 21(4), 164–167.

Berman-Frank, I.; Lundgren, P., and Falkowski, P., 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology,* 154, 157–164.

Bowen, J.L. and Valiela, I., 2001. The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Sciences,* 58, 1489–1500.

Cambareri, T.C. and Eichner, E.M., 1998. Watershed delineation and ground water discharge to a coastal embayment. *Ground Water,* 36, 626–634.

Capone, D.G. and Bautista, M.F., 1985. A groundwater source of nitrate in nearshore marine sediments. *Nature,* 313, 214–216.

Charette, M.A. and Allen, M., 2006. Precision ground water sampling in coastal aquifers using a direct-push, shielded-screen well-point system. *Ground Water Monitoring and Remediation,* 26, 87–93.

Charette, M.A.; Sholkovitz, E.R., and Hansel, C.M., 2005. Trace element cycling in a subterranean estuary, part 1: geochemistry of the permeable sediments. *Geochimica et Cosmochimica Acta,* 69, 2095–2109.

Coyer, J.A.; Cabello-Pasini, A.; Swift, H., and Alberte, R.S., 1996. N_2 fixation in marine heterotrophic bacteria: dynamics of environmental and molecular regulation. *Proceedings of the National Academy of Sciences,* 93, 3575–3580.

D'Avanzo, C.; Kremer, J.N., and Wainright, S.C., 1996. Ecosystem production and respiration in response to eutrophication in shallow temperate estuaries. *Marine Ecology Progress Series,* 141, 263–274.

Eyre, B.D.; Rysgaard, S.; Dalsgaard, T., and Christensen, P.B., 2002. Comparison of isotope pairing and N_2:Ar methods for measuring sediment denitrification—assumptions, modifications, and implications. *Estuaries,* 25(6A), 1077–1087.

Fisher, T.R.; Hagy III, J.D.; Boynton, W., and Williams, M.R., 2006. Cultural eutrophication in the Choptank and Patuxent estuaries of Chesapeake Bay. *Limnology and Oceanography,* 51, 435–447.

Fulweiler, R.W.; Nixon, S.W.; Buckley, B.A., and Granger, S.L., 2007. Reversal of the net dinitrogen gas flux in coastal marine sediments. *Nature,* 448, 180–182.

Gardner, W.S.; McCarthy, M.J.; An, S.; Sobolev, D.; Sell, K.S., and Brock, D., 2006. Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. *Limnology and Oceanography,* 51, 558–568.

Giblin, A.E.; Weston, N.B.; Banta, G.T.; Tucker, J., and Hopkinson, C.S., 2010. The effects of salinity on nitrogen losses from an oligohaline estuarine sediment. Estuaries and Coasts, 33, 1054–1068.

Glibert, P.M.; Kelly, V.; Alexander, J.; Codispoti, L.A.; Boicourt, W.C.; Trice, T.M., and Michael, B., 2008. In situ nutrient monitoring: A tool for capturing nutrient variability and the antecedent conditions that support algal blooms. *Harmful Algae,* 8, 175–181.

Glud, R.N.; Forster, S., and Huettel, M., 1996. Influence of radial pressure gradients on solute exchange in stirred benthic chambers. *Marine Ecology Progress Series,* 141, 303–311.

Herrera-Silveira, J.A. and Morales-Ojeda, S.M., 2009. Evaluation of the health status of a coastal ecosystem in southeast Mexico: Assessment of water quality, phytoplankton and submerged aquatic vegetation. *Marine Pollution Bulletin,* 59, 72–86.

Howarth, R.W. and Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. *Limnology and Oceanography,* 51, 364–376.

Hu, C.; Muller-Karger, F.E., and Swarzenski, P.W., 2006. Hurricanes, submarine groundwater discharge, and Florida's red tides. *Geophysical Research Letters,* 33, L11601, doi:10.1029/2005GL025449.

Jahnke, R.A. and Jahnke, D.B., 2000. Rates of C, N, P and Si recycling and denitrification at the US Mid-Atlantic continental slope depocenter. *Deep-Sea Research I,* 47, 1405–1428.

Jahnke, R.A.; Nelson, J.R.; Richards, M.E.; Robertson, C.Y.; Rao, A.M.F., and Jahnke, D.B., 2008. Benthic primary productivity on the Georgia midcontinental shelf: benthic flux measurements and high-resolution, continuous in situ PAR records. *Journal of Geophysical Research,* 113, C08022, doi:10.1029/2008JC004745.

Janssen, F.; Faerber, P.; Huettel, M.; Meyer, V., and Witte, U., 2005. Pore-water advection and solute fluxes in permeable marine sediments (I): calibration and performance of the novel benthic chamber system Sandy. *Limnology and Oceanography,* 50, 768–778.

Jones, K.N., 1982. Nitrogen fixation in the temperate estuarine intertidal sediments of the River Lune. *Limnology and Oceanography,* 27, 455–460.

Kana, T.M.; Darkangelo, C.; Hunt, M.D.; Oldham, J.B.; Bennett, G.E., and Cornwell, J.C., 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. *Analytical Chemistry,* 66, 4166–4170.

Kroeger, K.D. and Charette, M.A., 2008. Nitrogen biogeochemistry of submarine groundwater discharge. *Limnology and Oceanography,* 53, 1025–1039.

Lamontagne, M.G.; Astorga, V.; Giblin, A.E., and Valiela, I., 2002. Denitrification and the stoichiometry of nutrient regeneration in Waquoit Bay, Massachusetts. *Estuaries,* 25, 272–281.

Marino, R.; Chan, F.; Howarth, R.W.; Pace, M.L., and Likens, G.E., 2006. Ecological constraints on planktonic nitrogen fixation in saline estuaries, I: nutrient and trophic controls. *Marine Ecology Progress Series,* 309, 25–39.

Michael, H.A.; Mulligan, A.E., and Harvey, C.F., 2005. Seasonal oscillations in water exchange between aquifers and the coastal ocean. *Nature,* 436, 1145–1148.

Mulligan, A.E. and Charette, M.A., 2006. Intercomparison of submarine groundwater discharge estimates from a sandy unconfined aquifer. *Journal of Hydrology,* 327, 411–425.

O'Neil, J.M. and Capone, D.G., 1989. Nitrogenase activity in tropical carbonate marine sediments. *Marine Ecology Progress Series,* 56, 145–156.

Paerl, H.W., 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential. *Estuaries and Coasts,* 32, 593–601.

Paytan, A.; Shellenbarger, G.G.; Street, J.H.; Gonneea, M.E.; Davis, K.; Young, M.B., and Moore, W.S., 2006. Submarine groundwater discharge: an important source of new inorganic nitrogen to coral reef ecosystems. *Limnology and Oceanography,* 51, 343–348.

Presley, B. J., 1971. Techniques for analyzing interstitial water samples, part I: determination of selected minor and major inorganic constituents. *In:* Winterer, E.L.; Riedel, W.R.; Brönnimann, P.; Gealy, E.; Heath, G.; Kroenke, L.; Martini, E.; Moberly, R., Jr.; Resig, J., and Worsley, T. (eds.), *Initial Reports of the Deep Sea Drilling Project*. Volume 7. Washington, DC: U.S. Government Printing Office. pp. 1749–1755.

Rao, A.M.F. and Jahnke, R.A., 2004. Quantifying porewater exchange across the sediment-water interface in the deep sea with in situ tracer studies. *Limnology and Oceanography: Methods,* 2, 75–90.

Reddy, K.J.; Haskell, J.B.; Sherman, D.M., and Sherman, L.A., 1993. Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus Cyanothece. *Journal of Bacteriology,* 175, 1284–1292.

Schindler, D.W.; Hecky, R.E.; Findlay, D.L.; Stainton, M.P.; Parker, B.R.; Paterson, M.J.; Beaty, K.G.; Lyng, M., and Kasian, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences U. S. A.,* 105(32), 11254–11258.

Seitzinger, S.P., 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnology and Oceanography,* 33, 702–724.

Simonds, F.W.; Swarzenski, P.W.; Rosenberry, D.O.; Reich, D.D., and Paulson, A.J., 2008. Estimates of Nutrient Loading by Ground-Water Discharge into the Lynch Cove Area of Hood Canal, Washington. Reston, Virginia: U.S. Geological Survey Scientific Investigations Report 2008–5078, 54p.

Slomp, C.P. and Van Cappellen, P., 2004. Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. *Journal of Hydrology,* 295, 64–86.

Vitousek, P.M.; Cassman, K.; Cleveland, C.; Crews, T.; Field, C.B.; Grimm, N.B.; Howarth, R.W.; Marino, R.; Martinelli, L.; Rastetter, E.B., and Sprent, J.I., 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry,* 57/58, 1–45.

Weiss, R.F., 1970. Solubility of nitrogen, oxygen, and argon in water and seawater. *Deep-Sea Research,* 17, 721–735.