

Processing watershed-derived nitrogen in a well-flushed New England estuary

*Craig R. Tobias*¹

The Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02532

Matthew Cieri

Maine Department of Marine Resources, P.O. Box 8, McKnown Point Road, West Boothbay Harbor, Maine 04575

Bruce J. Peterson, Linda A. Deegan, Joseph Vallino, and Jeffrey Hughes

The Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02532

Abstract

Isotopically labeled nitrate ($^{15}\text{NO}_3^-$) was added continuously to the Rowley estuary, Massachusetts, for 22 d to assess the transport, uptake, and cycling of terrestrially derived nitrogen during a period of high river discharge and low phytoplankton activity. Isotopic enrichment of the 3.5-km tidal prism (150,000 m³) was achieved for the 3 weeks and allowed us to construct a nitrogen mass balance model for the upper estuary. Mean $\delta^{15}\text{NO}_3^-$ in the estuary ranged from 300‰ to 600‰, and approximately 75%–80% of the ^{15}N was exported conservatively as $^{15}\text{NO}_3^-$ to the coastal ocean. Essentially all of the 20%–25% of the ^{15}N processed in the estuary occurred in the benthos and was evenly split between direct denitrification and autotrophic assimilation. The lack of water-column ^{15}N uptake was attributed to low phytoplankton stocks and short water residence times (1.2–1.4 d). Uptake of water-column NO_3^- by benthic autotrophs (enriched in excess of 100‰) was a function of NO_3^- concentration and satisfied up to 15% and 25% of the total nitrogen demand for benthic microalgae and macroalgae, respectively. Approximately 10% of tracer assimilated by benthic autotrophs was mineralized and released back to the water column as $^{15}\text{NH}_4^+$. By the end of the study, ^{15}N storage in sediments and marsh macrophytes accounted for 50%–70% of the ^{15}N assimilated in the estuary. These compartments may sequester watershed-derived nitrogen in the estuary for time scales of months to years.

The inputs of anthropogenically derived nitrogen (N) to the oceans may now exceed inputs from natural sources (Howarth et al. 1996; Vitousek et al. 1997), and much of these loads pass through estuaries. Estuaries mediate the N flux between nitrogen-rich watersheds and nitrogen-limited coastal oceans and have been suggested as important sites for nutrient attenuation, cycling, and support of secondary production (Nixon et al. 1996; Chesney et al. 2000; Eyre 2000). Despite the importance of this link between fluvial and marine environments, there is currently an incomplete understanding of the mechanisms of N transformations, storage, and export within estuaries. Specifically, the extent to which biota in estuaries of differing trophic structure and hydrology use and/or attenuate watershed-derived nitrogen is only partially understood. Given the likelihood of increasing N loadings to estuaries, a better understanding of estuarine processing of N is critical to managing coastal zone resources.

Much has been learned from the construction of estuary-scale N budgets that combine bulk N measurements and hy-

drology, but insight into processing and identification of trophic linkages is limited using this approach (reviewed in Nixon et al. 1996). The relative importance of different N flow pathways must be inferred from the net changes in N content in various pools along the estuary. In general, budgets constructed from net changes in N stocks reveal limited information with regard to N turnover within pools or gross rates of transfer between pools.

The use of natural abundance stable isotopes has helped to better describe the flow of N through estuarine ecosystems, yet the overlap among isotopic end members and isotopic fractionation (particularly for nitrogen) can confound interpretation of natural abundance measurements (Deegan and Garritt 1997; Peterson 1999). Microcosm use of tracer levels of stable isotope (^{15}N) has provided better quantification of N cycling rates through dissolved inorganic, organic, and phytoplankton pools but at the expense of isolating these components of the ecosystem from natural hydrologic conditions (Lipschultz et al. 1986; Bronk et al. 1994). The potential “bottle effects” common to these types of incubations limit inquiry to organisms and processes that function on short time scales. Consequently they may not address N flow through higher trophic levels and effectively exclude possible synergies that may exist in the ecosystem.

The relatively new technique of in situ stable isotope enrichment has provided an increased understanding of N (and carbon) processing under natural conditions (Holmes et al. 2000; Hughes et al. 2000; Middelburg et al. 2000; Peterson et al. 2001; Tobias et al. 2001). In particular, whole ecosystem ^{15}N enrichments in streams have been instrumental in

¹ To whom correspondence should be addressed. Present address: U.S. Geological Survey, 431 National Center, 12201 Sunrise Valley Drive, Reston, Virginia 20192 (crtobias@usgs.gov).

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quantifying both N turnover via microbial processes (e.g., mineralization and nitrification; Wollheim et al. 2001) and N flow through food webs (Peterson et al. 1997; Tank et al. 2000). Because of increased water volumes and potentially large isotope losses due to tidal dispersion, the widespread application of whole-system isotope additions to estuaries has been limited. To date, a whole-estuary ^{15}N enrichment has been performed only once, in a New England estuary as part of the nitrogen isotope tracer experiment, (NISOTREX; Holmes et al. 2000; Hughes et al. 2000). The NISOTREX project demonstrated the utility of a whole-estuary ^{15}N enrichment, identified phytoplankton as the primary sink for watershed-derived N, quantified dissolved inorganic nitrogen recycling, and identified the role of phytodetritus in fueling the benthic food chain. This experiment described estuarine N processing under conditions of high water-column primary production when the rate of estuarine flushing was low.

These trophic and hydrodynamic conditions, however, were characteristic of only some estuaries. During periods of low phytoplankton production, especially in shallow estuaries, a significant portion of the total system primary production and autotrophic N demand is generated by benthic microalgae (MacIntyre et al. 1996). In addition to providing N transfer to higher trophic levels of the benthic food web (Heip 1995; Miller et al. 1996), benthic photosynthesis can accelerate the cycling and export of N through coupled nitrification/denitrification (An and Joye 2001). Given the potential importance of the benthic processes to the nitrogen biogeochemistry in estuaries, we seek a better understanding of estuarine N processing under non-phytoplankton bloom conditions.

The objective of this study was to assess the fate and transport of watershed-derived nitrogen (NO_3^-) during transit through a New England estuary under conditions of high river discharge, high benthic primary productivity, and low water-column primary productivity. Here we describe results from the NISOTREX II study, which employed the use of a long duration whole-estuary $^{15}\text{NO}_3^-$ enrichment in order to quantify N flow through multiple inorganic and biotic N pools within the Rowley estuary. This paper concentrates on N processing between inorganic pools and primary producers.

Methods

Site description—The Rowley estuary is located in north-eastern Massachusetts ($42^\circ44'\text{N}$, $70^\circ52'\text{W}$) within the Plum Island ecosystem long term ecological research site (PIELTER; Fig. 1). The 9-km estuary drains an approximately 17.2 km² mixed residential and forested watershed. The mean tidal amplitude is ~ 3 m, and the tidal excursion is 3–6 km depending on lunar tidal phase. The estuary discharges to the Plum Island Sound, which exchanges almost completely with the Gulf of Maine twice per day (Vallino and Hopkinson 1998). The estuary was divided into sampling stations at 0.5-km increments with station designations indicating the kilometer distance upstream from the mouth of Plum Island Sound (“k”). The study area for the whole-system isotope enrichment encompassed the upper 4 km of

the estuary (from 10.5k to 14.5k). High-water volume in this reach was 130,000 m³ with a benthic area of 50,000 m² (Fig. 2).

The entire estuary is bordered by extensive mesohaline and euryhaline marsh. Marsh macrophytes located in the upper 2 km of the estuary (12.5k–14.5k) consisted primarily of species of *Spartina*, *Carex*, *Scirpus*, and *Phragmites*. Regularly flooded *Spartina alterniflora* fringe marsh existed the length of the estuary and irregularly flooded *Spartina patens* high marsh dominated marsh flora downstream of 12k.

During the experiment, total estuarine primary producer biomass was dominated by two groups of benthic autotrophs: benthic microalgae (*Navicula* sp., *Nitzschia* sp.) and macroalgae (*Rhizoclonium* sp.). Benthic microalgae (BMA) were distributed primarily on sloping mudflats and in the channel bottom, while macroalgae (MACA) were restricted to the vertical marsh wall bordering the mudflats. Phytoplankton concentrations in the water column were consistently low ($<5 \mu\text{g}$ chlorophyll *a* [Chl *a*] L⁻¹), and the estuary was strongly net heterotrophic for the duration of the study.

Isotope addition—Two preliminary dye releases (1999 and 2000) and hydrodynamic simulations identified the upper estuary as the most suitable site for ^{15}N enrichment that would label the majority of the watershed NO_3^- inputs. The isotope addition site was located at 12.5k, approximately 2.5 km downstream from the site of nontidal freshwater input (15k). The enrichment solution consisted of the conservative tracer Rhodamine WT and K^{15}NO_3 (0.9 M, 10% atom enriched). The solution was dripped continuously into the water column using a metering pump at a rate of 20 g ^{15}N per day from 11 July to 2 August 2000 (22 d; 440 g ^{15}N total). Temperature, conductivity, and estuarine water level were recorded continuously at the drip site. River discharge at the head of the estuary was calculated from a stage-discharge curve generated from continuously monitored water levels and seasonal discharge measurements. These data were used to calculate advective fluxes into the study reach.

Field sampling—The stock size and isotopic enrichment of multiple N pools in both the water column and sediments were monitored prior to, during, and following the isotope addition. Sampling was conducted along the length of the estuary and occurred at 0.5- to 1-km intervals from station 8k to the freshwater river input end member located at the head of the estuary (station 15k). Sampling frequency of all pools ranged from 2- to 10-d intervals, with upstream stations (13k, 13.5k, and 14k) sampled every 2 to 6 d. All isotopic analyses were performed using either a Finnegan Delta S or Europa 20-20 isotope ratio mass spectrometer (IRMS) located at the Ecosystems Center, Marine Biological Laboratory. Enrichments were reported as $\delta^{15}\text{N}$ values in per million (‰) according to

$$\delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

where R_{sample} and R_{standard} were the $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard (‰ = 0), respectively.

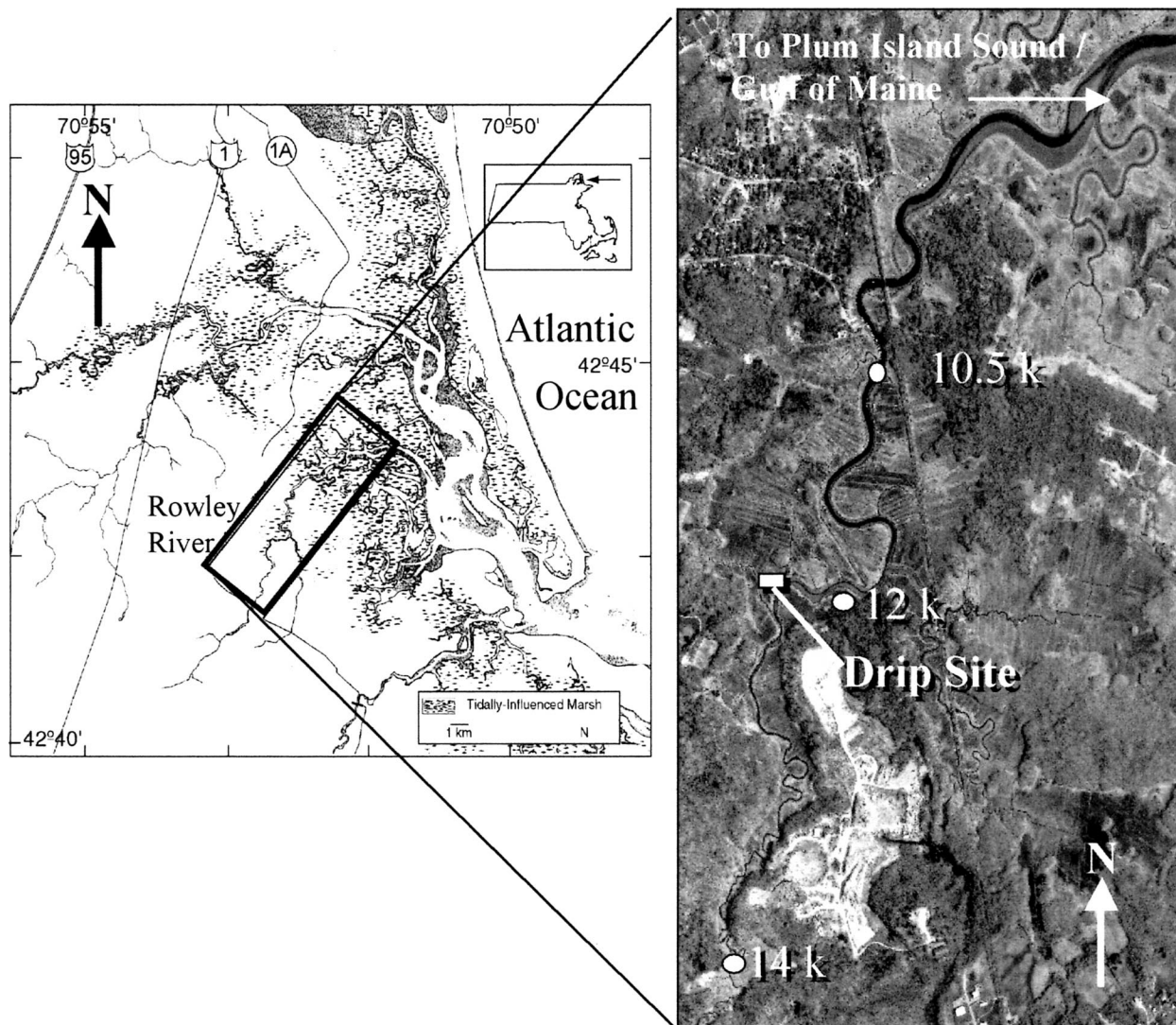


Fig. 1. Site location map and aerial photo. The ^{15}N drip site was located at 12.5k. The isotopically enriched reach extended from 10.5k to 14k.

Quantification of N stocks and enrichments—The water-column N pools quantified during the study included dissolved inorganic nitrogen (DIN; NO_3^- and NH_4^+), dissolved organic nitrogen (DON), and suspended particulate organic nitrogen (PON), which included both phytoplankton and suspended detrital nitrogen (DN). The benthic N pools quantified during the study included bulk sediment organic nitrogen (SED), benthic microalgae (BMA), macroalgae (MACA), and marsh macrophytes immediately fringing the estuary. ^{15}N tracer incorporation into higher trophic levels (pelagic and benthic) was also conducted and described in a separate paper (J. Hughes pers. comm.)

Water column—All samples for DIN and DON analyses were field-filtered ($0.7\ \mu\text{m}$, glass-fiber filter [GFF]) and frozen until analysis. NH_4^+ concentrations were determined using the indophenol method (Solorzano 1969). Nitrate ($\text{NO}_3^- + \text{NO}_2^-$) was measured spectrophotometrically using an Alpkem autoanalyzer following cadmium reduction to NO_2^- and

diazotization (Alpkem 1988). Total dissolved nitrogen (TDN) was quantified as NO_3^- following persulfate oxidation according to Valderrama (1981), and DON was calculated as the difference between TDN and DIN. Isotopic determination of NO_3^- was conducted using diffusion techniques (Sigman et al. 1997). Water samples were $0.7\text{-}\mu\text{m}$ filtered, treated with MgO to raise the pH above 9.7, and boiled to reduce sample volume and drive off NH_3 and labile DON. NO_3^- in the boiled samples was reduced to ammonia using Devarda's alloy (EM Science DX125-2). The resulting NH_3 was trapped on an acidified filter disk (sandwiched between Teflon membranes) during a 1-week incubation at 40°C . Isolation of DON for isotope analysis was achieved using ion retardation resins (Bronk and Glibert 1991). With the exceptions of humics (which are typically considered the more recalcitrant DON fraction), the resins do not appreciably alter the relative composition of the DON pool (Bronk and Glibert 1991; Fumitaka and Miyazaki 1999).

Bulk suspended particulate organic matter (POM), which

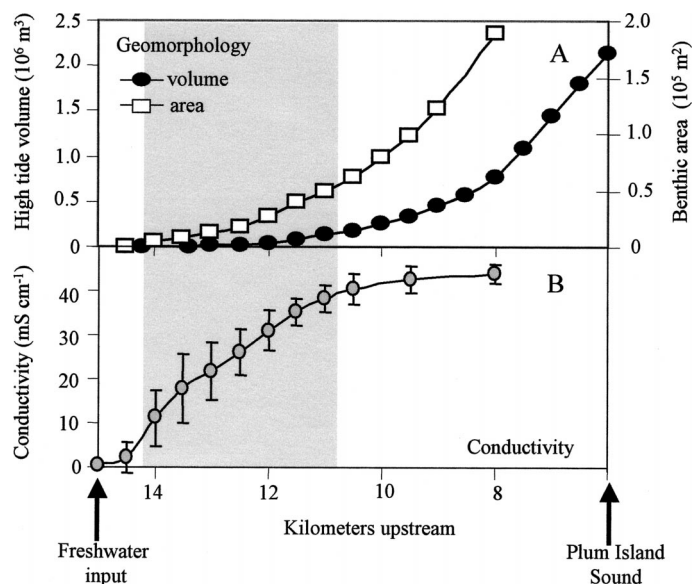


Fig. 2. (A) Estuary volume and benthic area. (B) Mean high-tide specific conductivity during the isotope enrichment period. Error bars denote standard deviation ($n > 6$). The shaded area represents the $^{15}\text{NO}_3^-$ enriched reach.

included phytoplankton and detritus, was filtered (0.7- μm GFF) from bulk water samples. Particulate organic nitrogen (PON) was determined from POM during IRMS analysis of the filters, and the N content was normalized to the filtered water volume to yield the PON concentration at each station. Phytoplankton-derived N was estimated from water-column chlorophyll concentrations (Strickland and Parsons 1972). The phaeophytin-corrected chlorophyll was converted to phytoplankton N equivalents by assuming a chlorophyll:carbon ratio of 50 and a phytoplankton molar C:N ratio 7, respectively (Redfield 1958). Because live phytoplankton were assumed to be the sole (or primary) sink within the PON pool (bacterial biomass represents a negligible fraction of the total PON mass) the phytoplankton enrichment ($\delta^{15}\text{N}_{\text{phyto}}$) can be approximated (for enrichments $< \sim 5,000\text{‰}$) from the bulk PON enrichment according to the following mixing equation:

$$\delta^{15}\text{N}_{\text{PHYTO}} = \frac{(\delta^{15}\text{N}_{\text{PON}}[N_{\text{PON}}]) - (\delta^{15}\text{N}_{\text{DN}}[N_{\text{PON}}]) + (\delta^{15}\text{N}_{\text{DN}}[N_{\text{PHYTO}}])}{[N_{\text{PHYTO}}]} \quad (2)$$

where $\delta^{15}\text{N}_{\text{PON}}$ and $\delta^{15}\text{N}_{\text{DN}}$ were the isotopic enrichments of the bulk PON and suspended detrital N pools (equal to the background $\delta^{15}\text{N}_{\text{PON}}$), respectively. $[N_{\text{PON}}]$, $[N_{\text{phyto}}]$, and $[N_{\text{DN}}]$ were the N concentrations of each of those pools, respectively.

Benthos—Bulk sediment organic nitrogen (sed) and benthic primary producer pools (BMA, MACA) were assessed in 3.2-cm diameter 2-cm-deep cores collected from the channel, mudflat, and marsh wall. The 2-cm depth was chosen based on previously measured sediment Chl *a* profiles throughout the PIE-LTER. Bulk N content and $\delta^{15}\text{N}_{\text{sed}}$ were

determined simultaneously during IRMS analysis of dried, ground sediments.

BMA and MACA stock sizes were calculated from benthic chlorophyll measurements (Lorenzen 1967) of the channel bottom, mudflat, and marsh wall. Conversion of sediment chlorophyll concentrations in the mudflat and channel to N content for BMA assumed a Chl *a*:C ratio of 35 and the mean measured C:N ratio of 7.1. The MACA-derived chlorophyll found in the marsh wall was converted to N equivalents using Chl *a*:C of 75 and a C:N ratio of nine (Atkinson and Smith 1983). BMA were isolated from sediments for isotopic analysis by capturing the migrating benthic diatoms on a 210- μm Nytex screen during low tide. Screens were washed with deionized water, resieved through 20- μm mesh, and filtered onto ashed 1.0 μm glass fiber filters. Microscopic examination of the collection revealed typically better than 90% purity in the diatom assemblage. We assumed that the BMA collected on the sediment surface had migrated from deeper sediments within hours of collection (Kingston 1999) and therefore possessed an isotopic enrichment representative of the whole BMA population in the upper 2 cm of sediment. Resulting BMA calculations in the flow model are indicative of this assumption and may represent generous estimates of BMA-N uptake if there was a significant disparity in enrichment between surface and deep BMA. $\delta^{15}\text{N}$ was measured on rinsed, dried (40°C), and ground MACA collected from the marsh wall.

Representing a potentially large N sink, marsh macrophytes (*Spartina alterniflora*) inhabiting the estuary edge were harvested during the study. The new roots and shoots were dried, ground, and quantified for N content and isotopic enrichment simultaneously during IRMS analysis.

Isotope balances—A total ^{15}N inventory and ^{15}N -flow balance for the entire isotope addition period was constructed for the uppermost 2 km of the Rowley (12k–14k) where the majority of isotopic label was found. The ^{15}N inventory summarized ^{15}N storage in each of the N pools at the end of the isotope addition period and assessed ^{15}N recovery in the measured pools relative to the total ^{15}N released. The inventory was generated using stocks and enrichments measured on or near the final day of the isotope addition period (2 August 2000). The tracer ^{15}N content in each pool was determined from the isotopic enrichment and the stock size according to

$$^{15}\text{N}_i = \left(\frac{\Delta\delta^{15}\text{N}_i}{1000} \right) \times 0.00366 \times N_i \quad (3)$$

where i represents the individual pool, $\Delta\delta^{15}\text{N}_i$ is the isotopic enrichment of each pool above background (‰), N_i is the stock size, and 0.00366 is the fraction of ^{15}N in the accepted N standard (atmospheric $\text{N}_2 = 0\text{‰}$). Stock sizes for all components dissolved or suspended in the water column were calculated from high-tide concentrations and scaled up based on high-tide estuarine volumes. Benthic stocks (BMA, MACA, SED) were calculated to a depth of 2 cm and scaled up based on relative area of channel, mudflat, and marsh-wall area. Both water column and benthic N stocks were

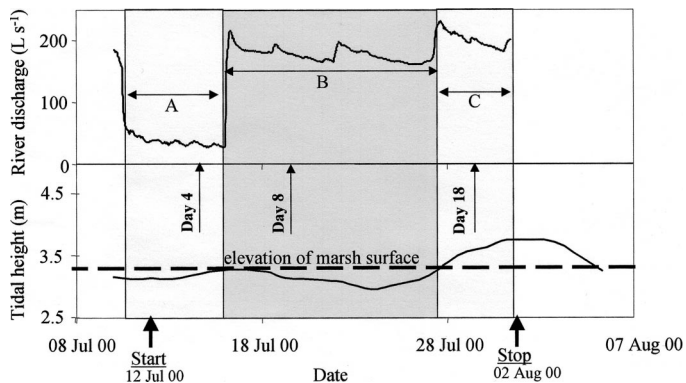


Fig. 3. Tidal and fluvial hydrology during the study. The isotope addition began on 12 July 2000 and ended on 2 August 2000. Letters A, B, and C correspond to sampling dates that capture one of the three distinct hydrologic conditions encountered during the enrichment period. (A) 16 July 2000: low discharge, small tidal input. (B) 20 July 2000: high discharge, small tidal input. (C) 30 July 2000: high discharge, large tidal input. Figs. 5, 7, and 8 show data sampled from all three hydroperiods.

calculated at 0.5-km intervals and summed to yield total N mass for each pool from 12k to 14k.

The ^{15}N -flow balance model summarized ^{15}N transfer from the labeled NO_3^- pool to primary producers, benthic regeneration, direct denitrification of $^{15}\text{NO}_3^-$, and ^{15}N export (as NO_3^-) from the estuary due to advection/dispersion. The flow model was constructed from data collected on 20 July 2000 (9 d into the enrichment experiment), which was representative of the hydrodynamic conditions prevailing for the majority of the experiment. ^{15}N transfer rates ($\text{g } ^{15}\text{N d}^{-1}$) from NO_3^- to primary producers were calculated from ^{15}N stock sizes and turnover times according to

$$^{15}\text{N}_{\text{uptake},i} = ^{15}\text{N}_{\text{max},i}u \quad (4)$$

where $^{15}\text{N}_{\text{max},i}$ is the ^{15}N stock ($\text{g } ^{15}\text{N}$) of the BMA, MACA, or phytoplankton, respectively, calculated during maximum observed enrichment and u is the specific uptake rate (d^{-1}). Specific uptake rates for BMA and MACA were estimated from the initial rise in the isotope trajectory at each station and defined as the slope of the isotope trajectory from a plot of $\ln \delta^{15}\text{N}_i$ versus time. The reciprocal of u was defined as the turnover time (τ). The specific uptake rate for phytoplankton was assumed to be 0.69 d^{-1} (i.e., $\tau = 1.4 \text{ d}$).

Recycling of ^{15}N from sediments back into the water column (as DIN) and direct denitrification of water-column $^{15}\text{NO}_3^-$ was estimated from core flux incubations reported in Tobias et al. (in press). Recycling and denitrification rates were scaled to the 12k–14k reach according to benthic area and daily tidal inundation duration.

The export rate of ^{15}N due to advection/dispersion was defined as the product of the water-column concentration of ^{15}N contained in all dissolved or suspended pools (NO_3^- , NH_4^+ , DON, PON) and the daily water volume export from the estuarine reach to Plum Island Sound. The mean concentration (volume weighted at 0.5-km intervals) of the water-column N pools was used in the export calculation. The daily water volume export (V_{out}) from the (12k–14k) reach to the Plum Island Sound was estimated from the single day

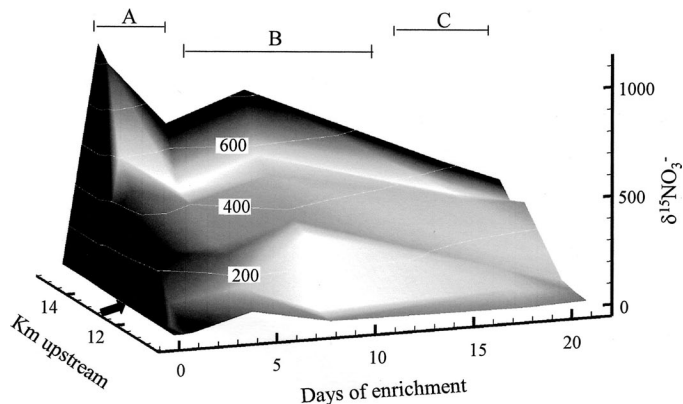


Fig. 4. Total $\delta^{15}\text{NO}_3^-$ enrichment inventory (high tide). The highest enrichments were measured furthest upstream (14k) and decreased downstream and over time due to higher fluvial and tidal inputs. Arrow indicates location of $^{15}\text{NO}_3^-$ dripper. A, B, and C denote the different hydroperiods as described in Fig. 3.

decrease in the estuarine rhodamine inventory after the dripper was stopped. V_{out} was calculated from

$$V_{\text{out}} = \left(\frac{\Delta \text{Rh}d}{\text{Rh}d_0} \right) \times V_{\text{total}} \quad (5)$$

where $\Delta \text{Rh}d$ was the single day change in high-tide rhodamine inventory contained in the reach between the last day of the drip and the first day following drip termination, $\text{Rh}d_0$ was the rhodamine inventory measured on the last high tide of the addition period, and V_{total} was the total water volume of the reach. The estimate of V_{out} was further constrained by two additional dye releases (August 1999, July 2000) and simulations from the Plum Island LTER estuarine hydrodynamic model (<http://ecosystems.mbl.edu/pie/data.htm>).

Results

Hydrodynamics—The isotope addition period was characterized by three distinct hydrodynamic conditions. During the first 4 d (Fig. 3A) river discharge (typically low in July) was 20 liters s^{-1} and accompanied by small tides that were insufficient to flood the surrounding marsh. The second hydroperiod (Fig. 3B) was characterized by high river discharge and small tides and represented the dominant hydrodynamic conditions during the isotope addition period. During the final week of the addition (28 July 2000–2 August 2000) high riverine discharge was accompanied by high spring tides that flooded the entire marsh (Fig. 3C). The rate of isotope addition ($20 \text{ g } ^{15}\text{N d}^{-1}$) was held constant during all three hydrodynamic regimes.

The $^{15}\text{NO}_3^-$ enriched reach encompassed the upper 3.5 km of the Rowley River and at high tide extended from 10.5k to 14k along the estuary (Fig. 4). Greater than 90% of the enrichment was detected in the upper 2 km of the estuary (12k–14k) characterized by a high-tide water volume of $\sim 46,000 \text{ m}^3$ and a benthic area of $\sim 24,000 \text{ m}^2$. Within this reach, the specific conductivity of the estuary ranged between 5 and 32 mS cm^{-1} (i.e., salinity = 3.3 to 22) and was highly variable as a function of river discharge, tidal stage,

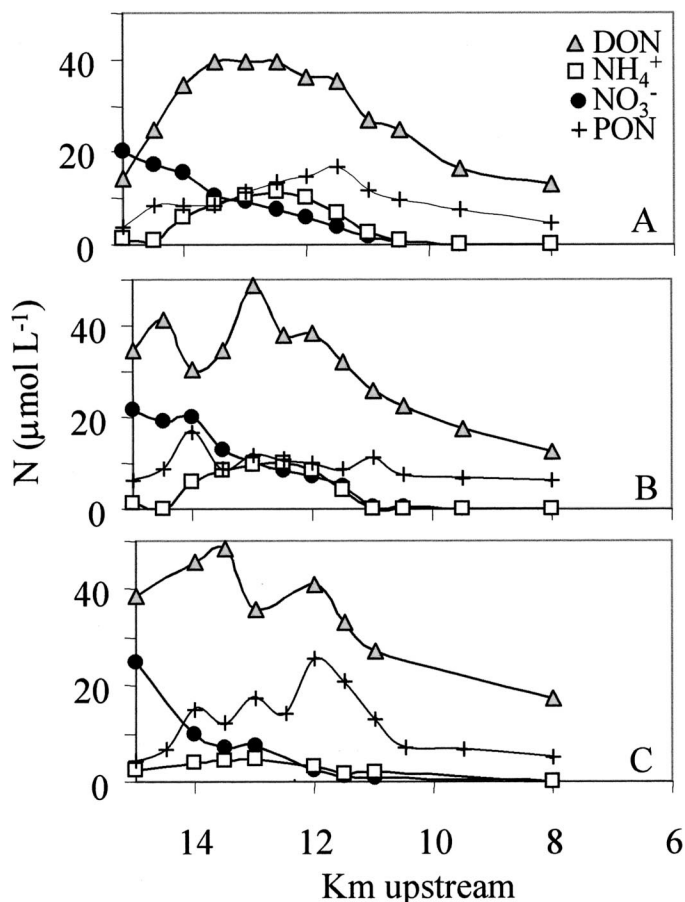


Fig. 5. High-tide spatial distribution of water-column dissolved nitrogen (DON, NH_4^+ , NO_3^-) and particulate nitrogen (PON). A, B, and C sampled on 14 July 2000, 20 July 2000, and 30 July 2000, respectively. Range of selected duplicates are smaller than the symbols.

and tidal phase (Fig. 2). Conductivity in the upper 2 km of the estuary was the most sensitive to hydrologic forcings where 10–15 mS cm^{-1} fluctuations during the isotope enrichment period were observed at individual stations. The estimate of water residence time in the 12k–14k reach, determined from three separate rhodamine dye releases, was 1.25–1.4 d. Approximately 75% to 80% of the high-tide water volume in this reach was exchanged daily.

Characterization of N pools—River input was the primary source of new NO_3^- to the estuary with a freshwater NO_3^- concentration ranging between 18 and 25 $\mu\text{mol L}^{-1}$ regardless of hydrodynamic conditions (Fig. 5). Total NO_3^- exported from the watershed to the Rowley during the 22-d isotope addition was 96 kg N, with an average daily flux for hydrodynamic periods A, B, and C of 0.43, 4.8, and 8.0 kg N d^{-1} , respectively. The daily drifter input rate of NO_3^- represented approximately 1% of the total NO_3^- stock in the reach and less than 5% of the daily riverine flux of NO_3^- . DON was the dominant nitrogen fraction in the water column with a concentration maximum ($>40 \mu\text{mol L}^{-1}$) in the upper estuary. In general, the watershed was also a source of DON, with concentrations decreasing downstream. How-

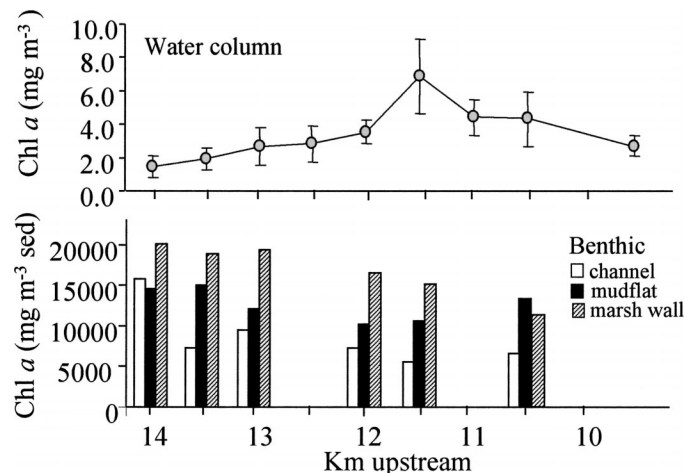


Fig. 6. Water-column (high tide) and benthic Chl *a* distribution during the enrichment period. Error bars for water-column concentrations are standard errors of all samples at each station ($n = 4$ or 6). The mean coefficients of variation for benthic chlorophyll at each station (all times; $n = 4$) for the marsh wall, mudflat, and channel were 0.32, 0.29, and 0.39, respectively.

ever some estuarine production of DON (particularly evident during low discharge periods; Figs. 3A, 5A) was observed. In contrast, the freshwater input was not a source of NH_4^+ to the Rowley estuary. Freshwater NH_4^+ concentrations were consistently below 2.0 $\mu\text{mol L}^{-1}$, and a middle estuary NH_4^+ maximum (up to 16 $\mu\text{mol L}^{-1}$) was observed in all transects except when there was large volume of saltwater intrusion during spring tides (Figs. 3C, 5C). The nonconservative behavior of NH_4^+ along the estuarine salinity gradient suggested that in situ production of NH_4^+ either through sediment desorption or mineralization (Hopkinson and Vallino 1995; Morlock et al. 1997; Hopkinson et al. 1999) was more important than watershed inputs in determining NH_4^+ distribution in the estuary. PON concentrations ranged from 5 $\mu\text{mol L}^{-1}$ measured at the freshwater and saltwater end members to a maximum of 18–30 $\mu\text{mol L}^{-1}$ in the middle estuary (between 12k and 10k; Fig. 5). While the maximum PON observed in the middle estuary ($\sim 12\text{k}$) may have resulted from a slightly higher phytoplankton content (Fig. 6), the mean phytoplankton N:PON ratio was 0.13 ± 0.02 (SE), indicating that $>85\%$ of the PON was not phytoplankton.

Water-column primary production was assumed to be low throughout the study due to the low phytoplankton stocks in the estuary during the whole study period. The time-averaged mean water-column Chl *a* concentration ranged from 2.0 to 8.0 mg Chl a m^{-3} in the upper 4 km of the estuary (Fig. 6). Whole-estuary oxygen metabolism transects (Hopkinson and Vallino 1995) indicated that the estuary was net heterotrophic during the entire study period. The dominant primary producer (as defined by total biomass) in the system was the benthic microalgae.

Benthic chlorophyll concentration (0–2-cm deep) was highest in the marsh wall dominated by the filamentous macroalgae (MACA) *Rhizoclonium* and increased upstream to 20,000 mg m^{-3} sed (400 mg m^{-2}) at station 14k. Benthic chlorophyll in the channel ranged between 6,000 and 15,000

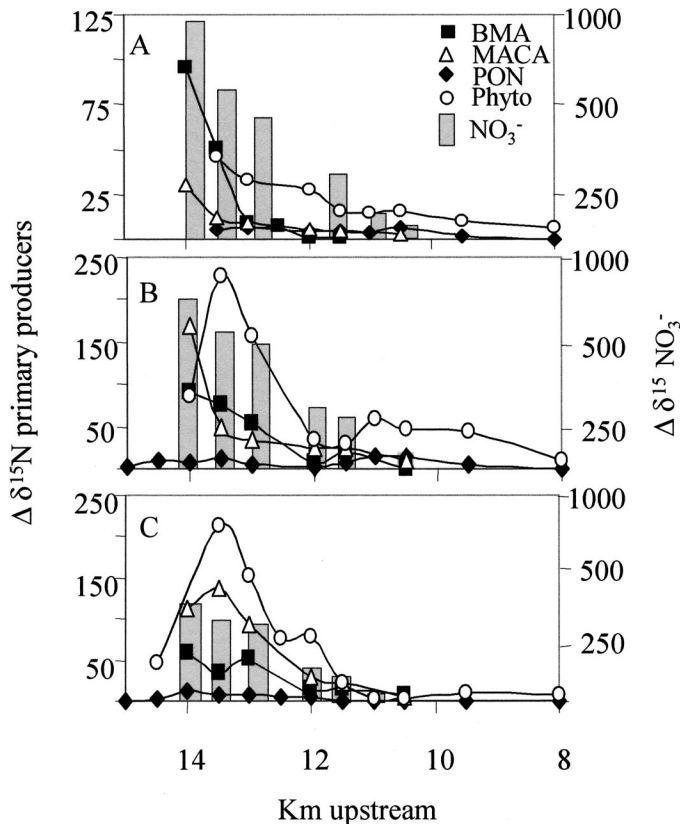


Fig. 7. Representative spatial distribution of isotopic enrichment (above background) for estuarine primary producers during the three hydroperiods (A, 13 July 2000; B, 21 July 2000; C, 01 August 2000). BMA, MACA, and Phyto are benthic microalgae, macroalgae, and phytoplankton, respectively. Isotopic enrichments of selected duplicates for BMA, MACA, PON, and NO_3^- were within 5% of the plotted means.

mg Chl *a* m^{-3} (120–300 mg m^{-2}) and in the mudflat 12,000–15,000 mg Chl a m^{-3} sed (240–300 mg m^{-2} ; Fig. 6). Total benthic chlorophyll decreased with distance upstream from 12k to 14k as a function of the decrease in total benthic area.

Isotopic enrichments— $\delta^{15}\text{NO}_3^-$ enrichment at high tide was detected from 10.5k upstream to 14k and, given the tidal excursion of 3.5–4 km, the enrichment extended to within 1 km of the mouth of the Rowley estuary at low tide (Fig. 4). Although the isotope was introduced at 12.5k, and despite the high NO_3^- concentration in the upper estuary (13–14k), peak $\delta^{15}\text{NO}_3^-$ enrichment (high tide) was measured at 14k (Figs. 4, 5). This high enrichment found at the head of the estuary occurred due to the smaller water volume and a generally lower rate of tidal dispersion found in upper estuarine reaches. $\delta^{15}\text{NO}_3^-$ enrichments dropped sharply upstream of 14k where the system was flooded with high concentrations of watershed-derived NO_3^- accompanying the freshwater discharge (Figs. 4, 5). Enrichment decreased gradually downstream as the labeled NO_3^- pool was increasingly diluted into larger water volumes and subject to shorter residence times resulting from the higher tidal dispersion downstream. Changes in the hydrodynamic conditions were evident in the

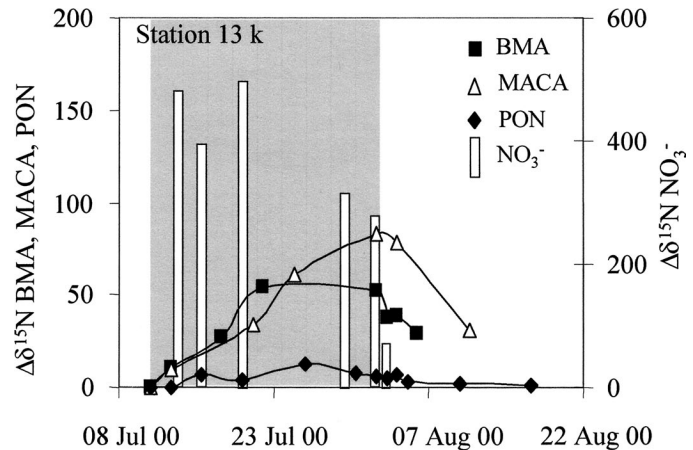


Fig. 8. Temporal distribution of primary producer enrichment at station 13k. The shape of the isotope trajectory (rise, plateau, and decline) was similar for all stations between 12k and 14k. The shaded area indicates the ^{15}N tracer addition period.

pattern of $\delta^{15}\text{NO}_3^-$ enrichment during the experiment. The initial increase in river discharge (16 July 2000) was accompanied by a sharp but temporary drop in the $\delta^{15}\text{NO}_3^-$ signal due to the downstream displacement of a large mass of the tracer (Fig. 4). However, the pattern of enrichment reached a new quasi-steady state with a peak $\delta^{15}\text{NO}_3^-$ at approximately 80% of the maximum observed during hydroperiod B (Figs. 3B, 4; high discharge; small tides). Despite the higher fluvial input of NO_3^- , this pattern of enrichment was maintained until the occurrence of spring tides, when the increased water volume (and N mass) and/or flooding the marsh (Figs. 3C, 4) caused a drop in $\delta^{15}\text{NO}_3^-$ of approximately 70% relative to the initial peak enrichments.

The highest directly observed enrichments in primary producers were found in the benthic autotrophs (MACA, BMA). Water-column PON showed little enrichment above background. With the exception of stations 11k–11.5k on 20 July 2000, PON enrichments above background ($\Delta \delta^{15}\text{N}$) were less than 5‰ (Fig. 7). The small maxima found at 11.5k on 20 July coincided with a slight chlorophyll maximum (Fig. 5; i.e., a higher proportion of the PON at 11.5k was composed of phytoplankton). Calculated phytoplankton enrichment maximums range from 50‰ to 250‰ and approximated the general pattern of increasing $\delta^{15}\text{NO}_3^-$ enrichment upstream in the estuary (Fig. 7). Despite the high enrichment calculated for the phytoplankton, water-column primary producers represented only a small sink for the ^{15}N tracer due to their small stock relative to the benthic autotrophs (Fig. 6). Enrichments in benthic primary producers also followed the spatial pattern in $\delta^{15}\text{NO}_3^-$ enrichment. Maximum MACA and BMA enrichments ($\Delta \delta^{15}\text{N}$) were found in the upper estuary (125‰ and 102‰, respectively) with a gradual decrease in enrichment downstream (Fig. 7). No enrichment above background was detected in BMA or MACA downstream of 10.5k. BMA in the upper estuary achieved maximum enrichment within 2–8 d following the start of the isotope addition, which was approximately 2–3 times faster than MACA (Figs. 7, 8, and 9A).

Mean turnover times (τ) for MACA (9.3 d \pm 1.9 [SD])

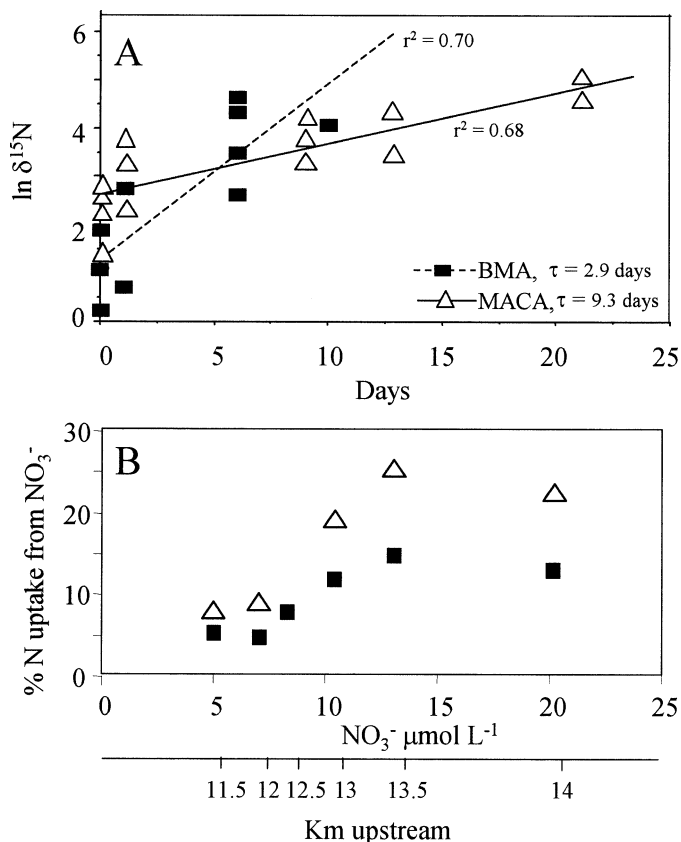


Fig. 9. (A) Estimation of turnover time for benthic microalgae and macroalgae using the transformed isotope trajectory data for all stations in the 12k–14k reach. Turnover time (τ) was equal to the inverse slope of the plot. (B) Estimation of the percent of the total N demand of each benthic autotroph (BMA and MACA) satisfied by the labeled NO_3^- pool. Percentage uptake from NO_3^- = (peak $\Delta\delta^{15}\text{N}_{\text{BMA,MACA}}$ /peak $\Delta\delta^{15}\text{NO}_3^-$) \times 100, where peak $\Delta\delta^{15}\text{N}_{\text{BMA,MACA}}$ is the peak primary producer enrichment (plateau of the isotope trajectory) and peak $\Delta\delta^{15}\text{NO}_3^-$ is the mean peak NO_3^- enrichment measured between the start of the enrichment and the occurrence of the peak primary producer enrichment.

and BMA ($3.0 \text{ d} \pm 0.8$ [SD]) for the four stations in the 12k–14k reach were calculated from the natural log transformed slope of the isotope enrichment trajectories (Fig. 9A). Despite the factor of two faster turnover time for BMA ($p < 0.025$, linear regression analysis of variance; Sokal and Rohlf 1981), peak MACA enrichment at all stations in the upper estuary was approximately 1.5 times higher than peak BMA enrichment. The peak enrichments of the benthic autotrophs relative to the $\delta^{15}\text{NO}_3^-$ values at each station indicated that direct NO_3^- uptake supplied 5%–15% and 5%–25% of BMA and MACA nitrogen demand respectively (Fig. 9B).

Recycling of ^{15}N tracer through biota and back into the dissolved N pool was assessed by measuring the $\Delta\delta^{15}\text{N}$ of NH_4^+ and DON pools. A maximal 15‰ enrichment in water-column $\delta^{15}\text{NH}_4^+$ was observed on 20 July and was coincident with the middle estuary NH_4^+ maximum (Tobias et al. in press). Core-based NH_4^+ flux measurements (Tobias et al. in press) scaled to the upper 2 km of the estuary indicated that

^{15}N was recycled from BMA to the overlying water at a rate of $0.15\text{--}0.2 \text{ g } ^{15}\text{N d}^{-1}$ (Fig. 10). While estuarine $\delta^{15}\text{N}$ -DON enrichments prior to the isotope addition decreased linearly ($r^2 = 0.95$) with distance upstream from 6.6‰ at 10.5k to 3.8‰ at 13k, no detectable $\Delta\delta^{15}\text{N}$ enrichment in DON was measured during the isotope addition period.

Storage of ^{15}N was measured at the end of the isotope addition period in marsh wall, channel, and mudflat sediments as well as in live marsh macrophytes. Bulk sediment contained the largest ^{15}N inventory (Table 1). Bulk sediments were enriched in ^{15}N almost 2 months after the ^{15}N addition had ceased. The highest enrichment was detected in the marsh wall of the upper estuary (9.5‰) and may reflect in part the more highly enriched MACA filaments in that sediment. $\Delta\delta^{15}\text{N}$ measured in mudflat sediments was highly variable, and the enrichment ranged from $<1\text{‰}$ to 5.9‰. In addition to sediments being a potentially large sink of ^{15}N tracer, *Spartina alterniflora* growing along the edge of the estuary from 13k–14k demonstrated enrichments in roots and young shoots of up to 20‰ and 9.7‰, respectively (Table 2). The isotopic enrichment first appeared in the roots and was followed by shoot enrichment within 1 week. Despite the relatively low $\delta^{15}\text{N}$ enrichments found in sediments and marsh macrophytes, these pools constituted a significant sink for ^{15}N mass due to their large mass of total N.

Discussion

The whole-estuary isotope enrichment approach provided the unique ability to examine N flow through multiple estuarine pools simultaneously while maintaining natural hydrologic and biogeochemical gradients and ecosystem synergies. The approach works best in small estuaries, particularly in tracing N through biotic components of the system. In general, the bulk of the N-flow information was derived from the isotopic rates of change between the pre-isotope addition steady state to the new isotope labeled steady state. Because different components of the ecosystem needed different lengths of time to reach the new isotopically labeled steady state, the isotopic rates of change represent different time-integrated measures dependent on the turnover time of the respective pool. This time integration should be kept in mind when considering the results and interpretation of the isotopically derived N transfer rates or N partitioning between different compartments in the estuary. Further, estuaries are seasonally dynamic, and thus the 3-week tracer addition presented here represents a snapshot in time that may or may not be representative of N processing mechanisms throughout the year. Nevertheless, the large-scale isotope approach was unique in that it provided experimentally based inquiry on kilometer-sized spatial scales and multi-week temporal scales.

The $^{15}\text{NO}_3^-$ addition to the Rowley estuary quantified the role of the benthos in processing watershed-derived N. The experiment specifically identified (1) the direct use and processing of water-column NO_3^- by benthic autotrophs; (2) the importance of benthic autotrophy relative to denitrification; (3) the potentially longer term (months–years) storage of processed N in the sediments and adjacent marsh macro-

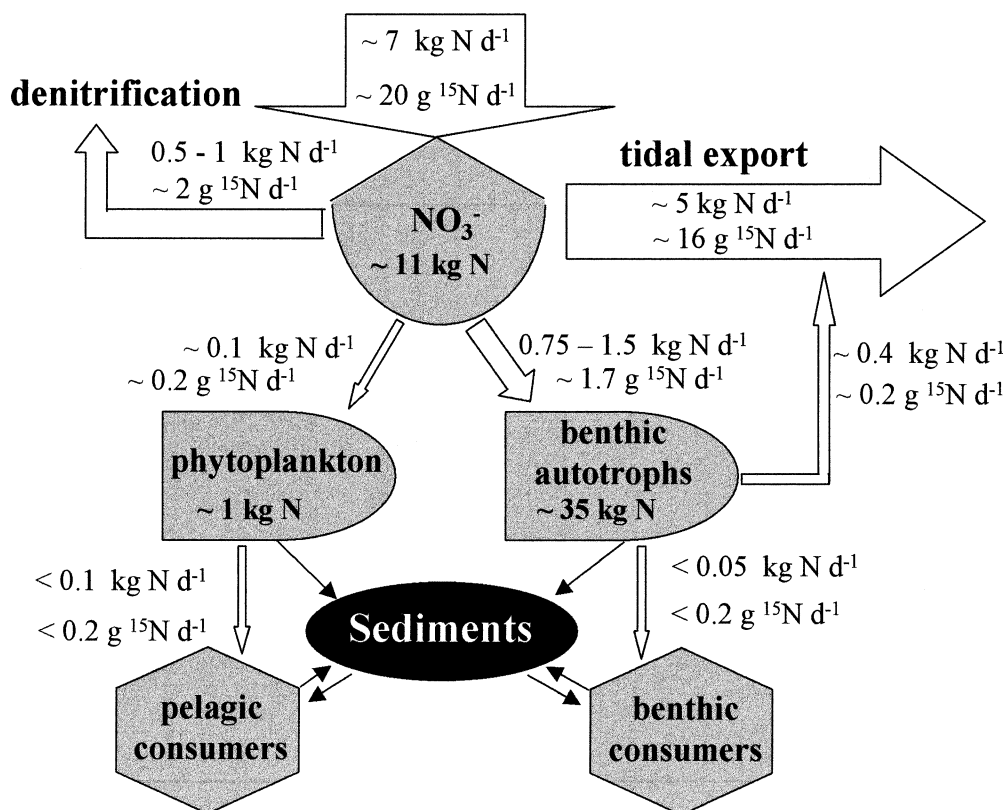


Fig. 10. Stocks and transfer rates of ^{15}N and total N for the upper estuary (12k–14k) calculated on 20 July 2000. Stations 12k–14k contained >90% of the total $^{15}\text{NO}_3^-$ enrichment inventory (high tide). The autotrophic biomass and production estimate does not include macrophytes in the adjacent marsh.

phytes. The tracer demonstrated that despite short residence times, water-column NO_3^- concentrations were high enough to significantly contribute to benthic N cycling.

^{15}N export and short-term processing—The immediate fate of the $^{15}\text{NO}_3^-$ was tidal export, uptake by benthic autotrophs, and direct denitrification (as extrapolated from core incubations; Tobias et al. in press). Approximately 75%–80% of the daily ^{15}N input to the estuary was exported to Plum Island Sound as NO_3^- , while approximately 18%–23% of the added $^{15}\text{NO}_3^-$ was processed within the estuary (Fig. 10).

Benthic processing (both autotrophy and denitrification)

was almost two orders of magnitude more important than pelagic sinks for the ^{15}N tracer (Fig. 10) and accounted for >95% of the ^{15}N processed in the estuary. The almost negligible role of phytoplankton N uptake in the Rowley River was in direct contrast to estuarine tracer studies conducted during periods of long water residence time and high water-column primary production (Holmes et al. 2000). Although the calculated phytoplankton enrichments in the Rowley were high (>200‰; Fig. 7), the phytoplankton biomass was so low that phytoplankton represented a sink for only 1%–2% of the added ^{15}N (0.1 kg total N d^{-1} ; Fig. 10) and supported little of the higher trophic production in the water column (J. Hughes pers. comm.). The small ^{15}N sink in phy-

Table 1. Sediment enrichments ($\Delta\delta^{15}\text{N}$). Mean ($\pm\text{SE}$; $n = 3\text{--}7$).

Mudflat	Sampling date in 2000				
	22 Jul	01 Aug	12 Aug	23 Aug	18 Sep
12k	0.6	1.7 (0.3)	0.3	—	0.0
13k	2.6 (1.1)	2.5 (0.9)	4.4 (0.1)	2.2	1.3 (0.2)
14k	2.8 (0.8)	3.5 (0.9)	5.0 (1.3)	5.4 (1.4)	4.1 (2.1)
Marsh wall					
12k	—	1.6 (0.2)	—	2.6 (0.4)	0.2
13k	—	6.0 (0.4)	2.5 (0.4)	2.9 (1.0)	4.1 (2.8)
14k	—	9.5 (3.0)	4.1 (1.7)	7.4 (1.1)	4.7 (1.7)

Table 2. *Spartina alt.* $\Delta\delta^{15}\text{N}$. Mean (\pm SE; $n = 3$).

Roots	Sampling date in 2000			
	22 Jul	02 Aug	10 Aug	23 Aug
13k	20.2	10.9 (0.8)	9.1	4.7
13.5k	21.8	8.5 (0.0)		
14k	11.0	9.6 (0.3)	4.6 (0.1)	8.3
Shoots				
13k	6.4 (0.2)	9.7 (0.8)	8.1	2.6
13.5k	3.0	4.3 (0.2)	4.4	
14k	0.0	5.7 (0.9)	5.7 (0.4)	6.7

toplankton was consistent with whole-estuary oxygen metabolism measurements (Hopkinson and Vallino 1995) that indicated low water-column primary production during the study.

Benthic autotrophy—If the water-column phytoplankton stock was generously assumed to turn over once per day, benthic autotrophy (BMA + MACA) still provided the majority of the total estuarine primary production. We calculated (based on conservative estimates of benthic algal stock and turnover time of 9 d) that the benthic autotrophs (BMA and MACA) in the Rowley River accounted for over 70%–75% of total primary production. This estimate compares favorably to other shallow macrotidal estuaries where benthic microalgae alone can be responsible for up to 50% of total system primary production (Underwood and Krokamp 1999).

High rates of benthic production generated a large benthic autotrophic N demand. By intercepting the flux of DIN from sediments to the water column, BMA are typically thought to assimilate nearly all of their N requirements from porewaters and are thus instrumental in mediating water-column DIN loads in estuaries (Rizzo 1990). In addition to retaining some porewater DIN that would otherwise be released to the water column, BMA and MACA directly assimilated labeled water column NO_3^- . The high enrichment observed in the BMA and MACA (100‰ and 175‰, respectively) exceeded that of the bulk sediment and sediment bacteria (Tobias et al. in press) by an order of magnitude and therefore could not have been achieved without the direct uptake of water-column nitrate. The higher peak MACA enrichment indicated that MACA satisfied more of its total N demand through the direct uptake of water-column NO_3^- than did BMA, and the difference was most pronounced at higher NO_3^- concentrations at the head of the estuary (Fig. 9B). The maximum $\delta^{15}\text{N}$ enrichment in the benthic autotrophs relative to the $\delta^{15}\text{NO}_3^-$ enrichment indicated that direct uptake of NO_3^- supplied 5%–15% and 5%–25% of BMA and MACA nitrogen demand, respectively. With the ratio of total N ($^{14}\text{N} + ^{15}\text{N}$) to ^{15}N label in the water-column NO_3^- was $\sim 500:1$, benthic autotrophy was a sink for approximately 11% of the daily NO_3^- inputs from the watershed (up to 19 kg total N- NO_3^- during the study). The ability to quantify the use of water-column NO_3^- represented an advancement over previous studies that could not distinguish sediment versus water-column sources of N contributing to the total N demand of

benthic autotrophs (Sundbäck and Miles 2000), and could help to further refine the calibration of benthic microalgal models (Cercio and Seitzinger 1997).

Benthic autotrophy versus denitrification—In contrast to uptake by benthic autotrophs, denitrification represented an immediate export of water-column NO_3^- out of the estuary (through evasion of N_2 and/or N_2O). A mean direct denitrification rate of water-column NO_3^- in the upper estuary during the NISOTREX II study of $3.5 \text{ mmol N m}^{-2} \text{ d}^{-1}$ was estimated by Tobias et al. (in press). When this rate was scaled to the 12k–14k estuarine reach and corrected for tidal inundation times, denitrification was nearly equal to the NO_3^- demand by benthic autotrophy. Each of these sinks was equivalent to approximately 10%–12% of the total daily NO_3^- flux from the watershed to the estuary.

Although recent studies in some macrotidal estuaries have suggested that denitrification was less important than benthic autotrophic N uptake in attenuating estuarine N loads (Sundbäck and Miles 2000; Cabrita and Brotas 2000), our results suggested that these two processes were equally important in the Rowley River (Fig. 10). It is possible that the scaled estimate of denitrification relative to benthic autotrophic uptake was generous because it did not consider the potential inhibition of direct denitrification by benthic oxygenic photosynthesis during illumination (Dong et al. 2000; Sundbäck and Miles 2000). Further, our estimate of autotrophic N uptake did not consider the potential magnitude of the sediment DIN flux intercepted by benthic autotrophs. Either of these scenarios would decrease the importance of denitrification relative to benthic autotrophy in total watershed N removal. However, the combined estimated magnitudes of benthic autotrophic N uptake and denitrification were consistent with overall ^{15}N mass balance.

Benthic ^{15}N recycling and storage—What was the fate of the approximately 9%–11% of the added ^{15}N that was assimilated by benthic autotrophs (Figs. 11, 12)? While this uptake represents a potentially important sink for water-column N during this study, the N was not directly removed from the marsh/estuary complex. The mean turnover time of BMA and MACA nitrogen was estimated at 3.0 and 9.3 d, respectively. These relatively short turnover times suggested that benthic autotrophs were potentially transient storage pools for the watershed-derived N. In other words, the assimilation by benthic autotrophs did not equate with instantaneous burial or with complete attenuation of watershed-derived N. One possible fate of the microalgal ^{15}N may have been tidal export following resuspension of BMA (Middelburg et al. 2000). Benthic diatoms, however, were not routinely observed in the water column, and unlike the prominent role of resuspension in controlling benthic microalgal populations in some higher energy estuarine mudflats (Middelburg et al. 2000), resuspension followed by tidal export was a relatively minor mechanism controlling the fate of BMA in the Rowley River. A more significant pathway of microalgal ^{15}N processing was mineralization and release of ^{15}N enriched NH_4^+ back to the water column (Tobias et al. in press). Once released back into the water column, the recycled $^{15}\text{NH}_4^+$ was subject to primarily export via the high rates of

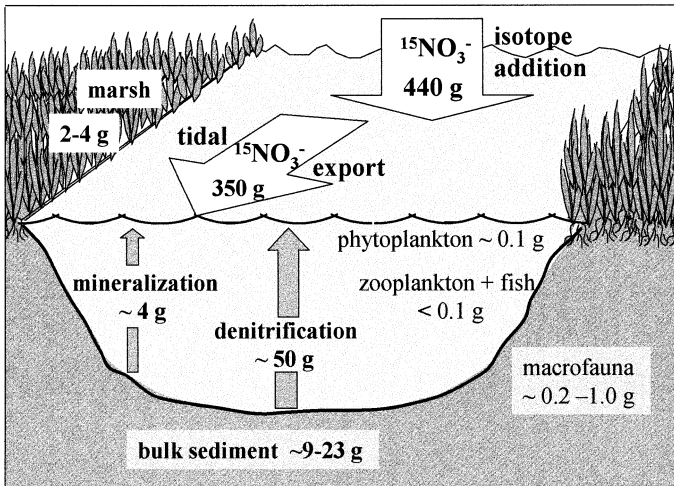


Fig. 11. ¹⁵N flow and storage scaled to the 22-d enrichment period. Flows are denoted with accompanying arrows. The 9 and 23 g of ¹⁵N storage in the bulk sediments assumed sediment mixing to a depth of 2 and 5 cm, respectively. The bulk sediment estimate includes ¹⁵N contained in BMA.

tidal exchange. Based on core incubations and the observed in situ $\delta^{15}\text{NH}_4^+$ enrichment (Tobias et al. in press), the release of ¹⁵N labeled NH_4^+ from sediments to the water column represented approximately 1% of the daily ¹⁵N addition or 11%–16% of the uptake rate of ¹⁵N from water-column NO_3^- by BMA (Figs. 10, 12). Sediment release of labeled DON may have also occurred, but DON enrichment was not detected due to higher background DON concentrations. Given the observed production of $^{15}\text{NH}_4^+$ in sediments, it is also plausible that an unknown amount of regenerated $^{15}\text{NH}_4^+$ was lost to coupled nitrification/denitrification. This process can be accelerated by benthic microalgal photosynthesis (Dong et al. 2000; An and Joye 2001) and may be sufficient to account for the small but unknown ¹⁵N sink in the ¹⁵N storage inventory constructed at the end of the study (Fig. 12).

Of the remaining ¹⁵N that was initially assimilated, less than 2% was stored in higher trophic levels (benthic infauna, epifauna, and benthivorous fishes; J. Hughes pers. comm.;

Figs. 10, 11). ¹⁵N enrichments of some benthic infaunal organisms were equivalent to BMA enrichments (J. Hughes pers. comm.) and demonstrated that the labeled BMA or MACA nitrogen fueled some benthic secondary production. However, stock sizes of the secondary producers were not large enough to generate a significant ¹⁵N storage term in the mass balance, and higher trophic levels played a minor role in N processing (Figs. 10, 11).

As observed in previous in situ tracer releases, bulk sediments retained the majority of the ¹⁵N excess inventory by the end of the isotope addition period (Figs. 11, 12). This pool is highly variable and difficult to characterize but represents a large N stock and the dominant fate of the watershed-derived N that is retained in the estuary. The average 6‰ enrichment of the upper 2 cm of bulk sediment on the final day of the isotope addition yielded a conservative estimate of sediment ¹⁵N storage of 9 g or approximately 24% of the ¹⁵N assimilated by benthic autotrophs (Fig. 12). However, sediment ⁷Be profiles indicated extensive downward mixing of sediments on monthly time scales (T. Dellapenna pers. comm.), which may have facilitated the burial of the ¹⁵N to depths of 5 cm. Recalculation of ¹⁵N stored in sediments based upon a 5-cm-deep sediment mixed zone would increase the estimated sediment ¹⁵N storage by a factor of 2.5, which would account for 60% of the ¹⁵N assimilated by benthic autotrophs over the duration of the isotope addition (Figs. 11, 12). The ¹⁵N label in the sediments, however, could have simply represented labeled benthic autotrophs mixed into the bulk sediment POM. Based on sediment chlorophyll concentrations, approximately 45%–60% of the ¹⁵N found in bulk sediments could be attributed to ¹⁵N contained in benthic autotrophs, which were actively being mineralized to DIN, released to the water column (Tobias et al. in press), and exported to Plum Island Sound. Nevertheless, bulk sediments did continue to display enrichment of >4‰ nearly 2 months after the end of the addition period, indicating that some of the tracer was retained in the sediment and potentially buried. Despite the insights of this study into understanding routes and rates of N processing through the use of ¹⁵N tracer, determination of burial on time scales exceeding 1–2 months was not possible given the low level of enrichment observed in the sediments. The percent of watershed-

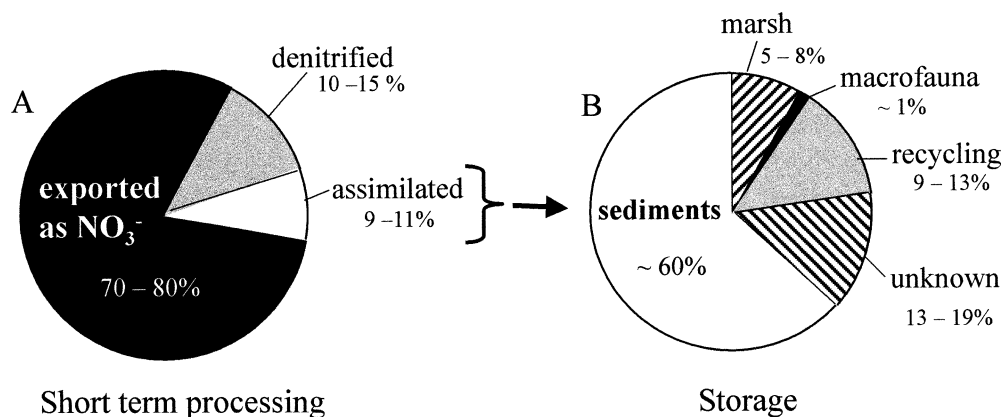


Fig. 12. (A) ¹⁵N processing presented as the percentage of ¹⁵N released. (B) ¹⁵N recycling and storage presented as a percentage of ¹⁵N assimilated.

derived N that becomes buried in estuarine sediments continues to be one of the greatest uncertainties in understanding how these ecosystems process, export, and sequester N on long time scales and should be a goal of future isotope enrichment studies.

The ^{15}N enrichment in creekbank *Spartina alterniflora* indicated a strong link between water-column nutrients and marsh production and another potentially large slow-turnover sink for N (Table 2). While the enrichment in roots preceded that in shoots, indicating that the roots were the point of uptake, the high sediment denitrification rates suggested that it was unlikely that direct root uptake could effectively compete for the $^{15}\text{NO}_3^-$ entering the sediments. Benthic algae (BMA and MACA), which were abundant on sediments between shoots, may have acted as an intermediate between water-column NO_3^- and plant uptake by assimilating labeled NO_3^- and recycling the ^{15}N tracer through the porewater NH_4^+ pool. Despite the uncertainties in tracing the route of ^{15}N uptake into the plants, the large size and long turnover time of the marsh macrophytes suggested their potential importance in longer term N storage. With conservative estimates of the width of the regularly flooded marsh (1 m) for only the uppermost 1 km (13k–14k) of the estuary (this area is less than 5% of the total marsh area along the 13k–14k reach) and *Spartina alterniflora* biomass estimates (<http://ecosystems.mbl.edu/pie/>), marsh macrophytes contained ~ 48 kg N. Given the average $\Delta\delta^{15}\text{N}$ enrichment of 14‰ in roots and 7‰ in shoots, respectively, 2–4 g ^{15}N was stored in *Spartina* at the end of the enrichment period. This mass was equivalent to 7% of the ^{15}N processed in the estuary and demonstrated an important linkage among watershed NO_3^- , estuarine water-column DIN, and marsh N storage (Fig. 11). Similar to the sediment storage, the duration of N retention/burial in the marsh remained unclear. Marsh samples collected in December 2000 (4 months after ^{15}N addition) showed no discernible pattern of enrichment; however, the large mass of N within the *Spartina* may have simply diluted any remaining excess ^{15}N to levels below detection. Reported *Spartina* turnover times of 8 months (above ground; Morris and Haskin 1990) to 3 yr (below ground biomass; Blum 1993) suggest that any ^{15}N stored in the macrophytes at the end of the experiment would remain in the marsh on the scale of months to years.

Linking seasonal hydrography to benthic/pelagic N processing—The benthos was the dominant site of watershed N processing in the Rowley due to hydrologic forcings on water-column NO_3^- concentration (10–20 $\mu\text{mol L}^{-1}$) and on water residence time (<2 d). Rates of NO_3^- removal from the water column by benthic autotrophs and denitrification were proportional to the NO_3^- concentrations (Fig. 9B; Tobias et al. in press), and the water-column NO_3^- concentrations were controlled by a feedback between the water residence time and the biota in the estuary. Unseasonably high river discharge supplied a large flux of NO_3^- rich water to the estuary and decreased the water residence times to levels that were too short to support high phytoplankton biomass. The resulting low phytoplankton demand for NO_3^- in the water column in turn allowed for the maintenance of higher NO_3^- concentrations, which in turn fueled benthic processing. We

suggest that benthic processing of watershed-derived NO_3^- would be optimal when hydrologic forcings (fluvial discharge and tidal dispersion) generate the longest possible water residence times that do not exceed phytoplankton doubling times. Pelagic N processing would dominate at longer water residence times when phytoplankton biomass could accumulate. Despite the high efficiency of allochthonous N uptake in phytoplankton blooms (e.g., uptake of 100% of the watershed N loading; Holmes et al. 2000), the estuaries entering the Plum Island Sound experience only short periods (2–3 months yr^{-1}) of low fluvial discharge and elevated phytoplankton biomass. Consequently the high phytoplankton uptake rates operate only for a small portion of the year, and the lower rates of benthic N processing operate for the majority of the year. When the rates of pelagic (phytoplankton) and benthic (denitrification and autotrophy) N processing are integrated over an annual cycle, benthic processing amounts to a larger sink for watershed N than phytoplankton uptake by a factor of 1.2. In estuaries similar to those of the Plum Island Sound system, which experience only short duration transient phytoplankton blooms, total annual processing of watershed N in the estuary may occur primarily through benthic pathways rather than via phytoplankton assimilation. When combined with previous whole-estuarine isotope enrichment research (Holmes et al. 2000; Hughes et al. 2000), this study illustrated interactions between hydrology, N loading, and ecosystem structure in regulating watershed-derived N cycling in estuaries.

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