Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication

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ABSTRACT: The bioavailibility of dissolved organic nitrogen (DON) in river water entering estuaries was examined for the Delaware and Hudson Rivers, USA. Water collected from above the salinity intrusion zone of each river was filtered, brought to a salinity of 15 ppt, and inoculated with estuarine bacteria. Bacterial production rates (8 to 26 × 10⁵ cells ml⁻¹ d⁻¹) during the initial 2 d in these experiments were within the range measured in these and other estuaries, indicating that riverine dissolved organic matter can contribute to production of estuarine bacteria. Average DON concentrations decreased by 40 to 72% within the 10 to 15 d time course of the experiments; the decreases in DON were accounted for by increases in microbial biomass plus remineralization to inorganic nitrogen. The time scale over which DON was utilized suggests that in estuaries with residence times on the order of weeks to months, such as Delaware Bay, river inputs of the biologically available portion of DON are first utilized within the estuary. In contrast, in estuaries with residences times of less than a week, such as New York Bay, a portion of the biologically available DON may be utilized first within the estuary, with the remainder exported and utilized in continental shelf waters. The large proportion of the DON that was biologically available in these experiments, coupled with the knowledge that inputs of organic nitrogen can account for 20 to 90% of the total nitrogen loading to estuaries, suggests that organic nitrogen inputs may contribute more to estuarine and shelf eutrophication than was previously suspected. These experiments demonstrate that dissolved inorganic nitrogen (DIN) inputs underestimate, and total nitrogen inputs likely overestimate, the inputs of biologically available N to estuaries. In order to develop a 'biologically available nitrogen budget' for an ecosystem, DIN inputs, plus that portion of the organic N that is biologically available must be quantified.

KEY WORDS: Dissolved organic nitrogen · Nitrogen · Estuaries · Rivers · Microbial processes · Eutrophication

INTRODUCTION

If the current and future eutrophication of estuaries and near shore marine waters is to be ameliorated, a clear understanding of the external sources of nutrients, their effect on the ecosystem, and their removal from the ecosystem is essential. Decreasing nitrogen (N) inputs is especially important because N is the nutrient that is most limiting to primary production in many estuarine and coastal waters (Ryther & Dunstan 1971, Oviatt, et al. 1995). Most studies of nutrient inputs to estuaries have examined the response to dissolved inorganic N (DIN = ammonia, nitrate, nitrite), because these forms of nitrogen are known to be incorporated rapidly by phytoplankton and to contribute to eutrophication (Ryther & Dunstan 1971, Boynton et al. 1982, D'Elia et al. 1986, Howarth 1988). However, DIN accounts for only a portion of the nitrogen inputs. The effect of organic N, which comprises the remainder of the loading, has been largely ignored.

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Both particulate and dissolved forms of organic nitrogen contribute to estuarine N loading, but the dissolved forms are most likely to be immediately available to planktonic microorganisms. Bacteria and some phytoplankton rapidly assimilate very low molecular weight compounds, such as amino acids and urea (e.g. Wheeler & Kirchman 1986, Fuhrman 1990, Antia et al. 1991, Glibert et al. 1991, Keil & Kirchman 1991, Cotner & Gardner 1993, Jorgensen et al. 1993, Tranvik 1993). These low molecular weight compounds are generally considered to comprise 20% or less of the dissolved organic nitrogen (DON) transported by rivers to estuaries, with the bulk of the DON consisting primarily of uncharacterized, complex, high molecular weight (HMW, >1 kDa) compounds (Thurmann 1985).

Traditionally, most dissolved organic matter (DOM) inputs to estuaries have been considered refractory and of limited biological availability based on the predominance of HMW compounds, and on findings that DOM mixing curves in some estuaries showed conservative behavior with respect to salinity (e.g. Mantoura & Woodward 1983). However, the utilization of nitrogen contained in the HMW compounds that make up the bulk of DON has not been substantially addressed by experimental measurements; studies have primarily focussed on the fate of C in DOM. Recent studies in the Amazon River and in a river entering the Gulf of Mexico support previous conclusions that 70% or more of the dissolved organic carbon (DOC) in rivers is contained in HMW compounds (Amon & Benner 1994, Santschi et al. 1995). In contrast to previous conclusions about the bioavailability of HMW DOC, recent studies demonstrate that a considerable portion of HMW DOC in rivers is readily used by bacteria (Meyer et al. 1987, Amon & Benner 1994). While studies such as these are changing our concepts about the fate of C in DOM, little is known about utilization of N in river inputs of DOM to estuaries. The fate of N in DOM can differ considerably from that of C depending on a number of factors, including the C:N ratio of the compounds utilized relative to the C:N ratio of bacterial consumers of DOM (Goldman et al. 1987, Gardner et al. 1996).

In order to fully understand the contribution of rivers to estuarine eutrophication, we need to know whether the influx of river transported organic N is biologically available. Organic N can be incorporated into the biological cycle in estuaries when microbial populations assimilate the N into bacterial biomass and/or regenerate the organic N as ammonia (Goldman et al. 1987, Caron & Goldman 1990). DON may also become biologically available due to the release of ammonia following photochemical oxidation of DON (Bushaw et al. 1996), degradation of DON by phytoplankton exoenzymes (Palenik & Morel 1990, Pantoja & Lee 1994) and
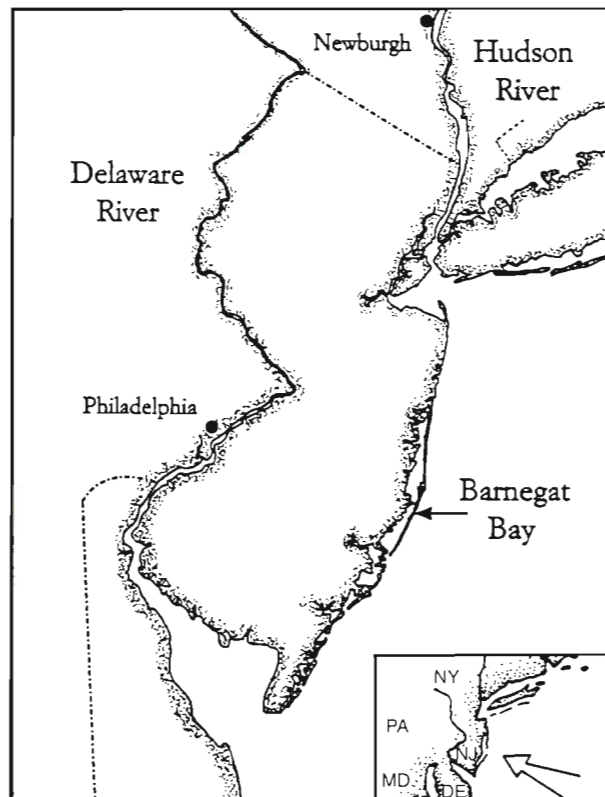

Fig. 1 Location of the Delaware and Hudson River sampling sites and the Barnegat Bay estuary in the northeastern U.S. All experiments used bacterial innocula from the Barnegat Bay estuary. Samples of river water for dissolved organic nitrogen (DON) experiments were collected just above the salinity intrusion zones of each estuary (Philadelphia, Pennsylvania, and Newburgh, New York)

by direct phytoplankton uptake (Antia et al. 1991, Paerl 1991). The present study examined how much of the DON in 2 rivers was readily utilized by natural assemblages of heterotrophic estuarine microbes, and the degree to which the DON was incorporated into cell biomass or regenerated as ammonia.

METHODS

Study sites. Utilization of riverine DON by estuarine microorganisms was studied using water from the Delaware and Hudson Rivers, USA. River water was collected just above the salinity intrusion zone of each estuary to represent DON actually reaching the estuary (Fig. 1). The Delaware was chosen because there are numerous point sources of organic matter (e.g. sewage treatment plants in the metropolitan Philadelphia area) that discharge just upstream of the salinity intrusion zone. This contrasts with the Hudson River where there are not major point sources in the region

near the salinity intrusion area (the New York City inputs enter within the saline portion of the estuary), but significant runoff from agricultural and forested areas along the length of the Hudson (Howarth et al. 1991, Clark et al. 1992).

Experimental setup. Two experiments were conducted to examine utilization of riverine DON by estuarine bacteria: an initial experiment in April 1992 with Delaware River water, and a second experiment in June 1992 with Delaware and Hudson River water to compare utilization of DON from the 2 rivers. Hereafter, the April and June experiments will be referred to as Expts I and II, respectively. The overall design of the experiments was to add concentrated natural assemblages of estuarine bacteria to sterile-filtered river water (after increasing the salinity to 15 ppt). This left DOM from the rivers as the primary organic compounds available for growth. Changes in microbial abundances and inorganic and organic N concentrations were then measured over time.

River water was collected in acid-cleaned and propanol-rinsed carboys. Carboys were rinsed with river water from the respective sites at the time of sample collection. During transport and processing the water was kept at 4°C. In the laboratory, river water was immediately filtered, first through 24 μm and then through 0.2 μm spiral-wound glass fiber filters to remove microorganisms and other particles. Based on repeated checks, there were no measureable changes in DOC or DON concentrations in river water as a result of this filtering procedure. A combination of pre-combusted salts (Kester et al. 1967) was added to establish an estuarine salinity of 15 ppt ($MgCl_2$ was not precombusted). Control water (Barnstead Nanopure water) was filtered and salts were added, using the same procedures employed for the river water.

Concentrates of estuarine bacteria were obtained using water (15 ppt) from a small estuary, Barnegat Bay, New Jersey, which is equidistant from the mouths of the 2 rivers (Fig. 1). We chose to use bacteria from Barnegat Bay, in contrast to bacteria from each river's estuary, so that differences in DOM utilization would reflect differences in organic matter among the sites, and not differences in bacterial populations. Barnegat Bay water was filtered through a 35 μm mesh plankton net and a 1 μm Nuclepore polycarbonate filter to remove larger organisms and particulates. Bacteria in the filtrate were then concentrated using either a 0.2 μm Nuclepore membrane filter (Expt I) or a 1 MDa polysulphone filter (tangential flow ultrafiltration; Filtron Technologies) (Expt II).

In Expt II a sonication step was introduced which eliminated remaining protists without significantly affecting bacterial growth (data not shown). The bacterial concentrate was prepared as above (including pre-

filtration) and then pulse sonicated using a Branson Cell Disruptor II with a microtip probe. Three batches of 33 ml each were sonicated over ice at the microtip power limit and a 50% duty cycle for 2.5 min. Preliminary experiments indicated that this step eliminated protists without significantly affecting bacterial growth at 24 and 48 h. Protists were observed after 24 h when shorter sonication steps were used, while longer steps appeared to inhibit bacterial growth.

Incubations. Bacterial concentrates (~18 ml) from Barnegat Bay were added to 10 l of river and control water to give an initial abundance in the treatment water of 8 to 11×10^5 cells ml^{-1} (Expt I) and 2 to 4×10^5 cells ml^{-1} (Expt II). Initial samples for nutrients and bacteria were taken from the river and control waters and then 4 l of each treatment water were poured into duplicate 4 l Erlenmeyer flasks (i.e. 2 control flasks and 2 flasks for each river with 4 l per flask). The flasks were capped with aluminum foil and incubated in the dark at 20°C; the water was stirred gently with Teflon coated stir bars.

Measurements. Time series samples of water were taken over a 23 d period during Expt I and over a 10 d period during Expt II (see results for exact sampling frequency). Water samples for DIN (NH_4^+, $NO_2^- + NO_3^-$) (Parsons et al. 1984, Alpkem 1991), dissolved reactive phosphate (DIP; Parsons et al. 1984), total dissolved N (TDN; Walsh 1989), and particulate N (PN; Expt II only) (Carlo Erba Elemental Analyzer) analysis were filtered through (dissolved) or collected on (particulate) pre-combusted (500°C) glass fiber filters (Whatman GF/F). DON was determined by the difference between TDN and DIN. TDN samples were analyzed by high temperature combustion followed by chemiluminescent detection of nitric oxide (Walsh 1989) using an Antek Model 7000 Total N Analyzer (Antek, Inc.) equipped with a quartz combustion tube ($1000 \pm 10°C$) and a ceramic insert. TDN samples were preserved in capped autosampler vials with 3 N HCl (7.5 μl acid per 1.5 ml sample). Blanks consisted of deionized distilled water. Both inorganic (ammonia and nitrate plus nitrite) and organic (urea) standards for TDN analysis were prepared in deionized distilled water.

Samples were analyzed for bacterial abundance (Francisco et al. 1973, Hobbie et al. 1977) and biovolume (Lee & Furhman 1987). Heterotrophic flagellate (HNAN) abundance and biovolume were determined using epifluorescent microscopy. The abundance and size of bacteria on glass cover slips suspended in the incubation flasks were examined to estimate growth on the flask walls (Hagström et al. 1984). Specific growth rates and doubling times of bacteria were calculated from changes in abundance during the first 2 d of the experiments. This was possible because predation-related mortality was eliminated in Expt II, and

flagellates had not yet reached abundances that would significantly impact bacterial populations in Expt I. Bacterial biovolumes were converted to C and N biomass using a conversion factor of 220 fg C μm^{-3} and a C:N ratio of 4.29:1 by weight (Bratbak 1985, Goldman et al. 1987). Flagellate biovolume was converted to biomass assuming 350 fg C μm^{-3} and a C:N ratio of 5.6:1 by weight (Sanders et al. 1996).

RESULTS

Experiment I

Average DON concentrations in the Delaware River water decreased rapidly in the first 3 d and then decreased more slowly through Day 15 (Fig 2A). By Day 4, initial concentrations (12.9 µM) had decreased by 63% to 4.7 µM; by Day 15 they had further decreased to 3.6 µM, or by 72% of the initial concentration (Table 1). Rates of DON utilization increased from 11.5% d^{-1} (1.5 µmol DON-N d^{-1}) during the first 24 h, to 42% d^{-1} (3.6 µmol DON-N d^{-1}) between Days 3 and 4 (Fig. 3). Rates then decreased to less than 5% d^{-1} (0.1 µmol DON-N d^{-1}) for the remainder of the experiment. DON concentrations in control water remained below 3 µM throughout the experiment, demonstrating that DON contained in the bacterial concentrate added at the beginning of the experiment was minimal and could not account for the pattern of DON decrease observed in the river water. DIP and DIN concentrations remained relatively constant throughout the experiment in both Delaware River water (DIP 2 µM; NH_4^+ 20 µM and $NO_3^- + NO_2^-$ 80 µM) and controls (NH_4^+ 0.2 µM and $NO_3^- + NO_2^-$ 0.2 µM).

Bacterial abundances in the Delaware River water increased rapidly during the first 2 d and then decreased to near initial levels on Day 4 (Fig. 2B). Production of bacteria during the first 2 d was estimated at 21×10^5 cells ml^{-1} d^{-1} based on changes in abundance (Table 2). Although HNAN initially were not observed, a few apparently were introduced in the inocula. The decrease in bacterial abundance in the Delaware River water on Day 4 coincided with an increase in the abundance of bacterivorous flagellates (Fig. 2C). Bacterial abundance rebounded by Day 7, after which both bacterial and flagellate abundances decreased to control levels and remained relatively stable until the end of the experiment. Bacterial community biovolume showed a similar pattern to the abundance because average individual biovolume of bacterial cells increased after Day 1 and remained similar for the rest of the experiment. Abundances and individual biovolumes of HNAN peaked on Day 7. By Day 7, the sum of the calculated bacterial and HNAN carbon in biomass
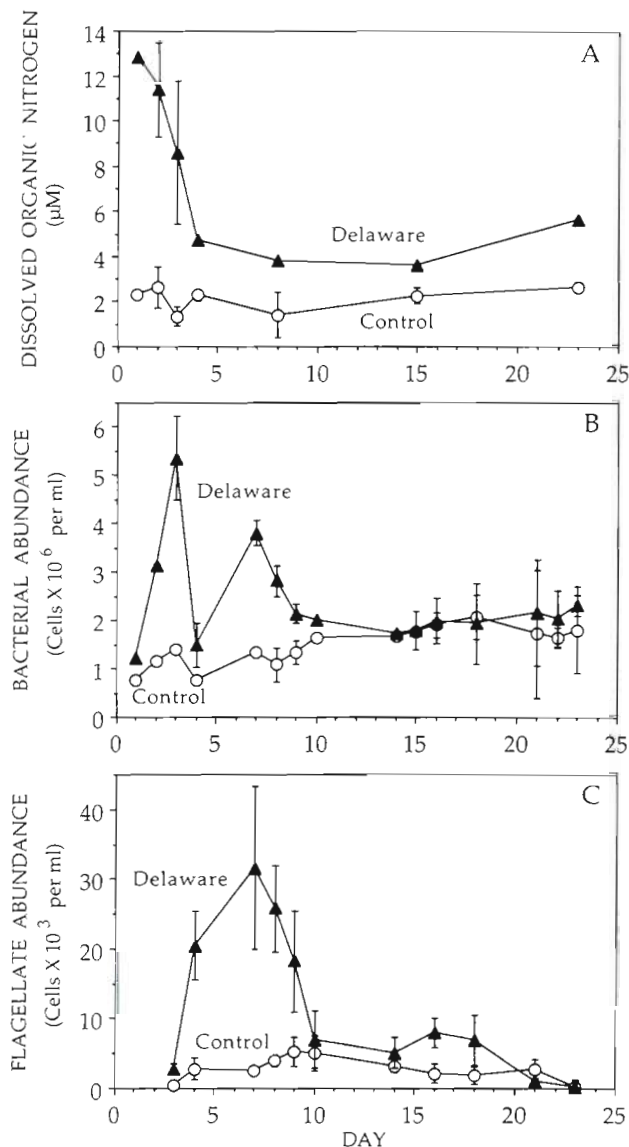

Fig. 2. Expt I. Time course of changes in (A) concentration of dissolved organic nitrogen, (B) bacterial abundance, and (C) heterotrophic flagellate abundance in Delaware River water (▲) and controls (○). Data represent the means of 2 replicate experimental chambers ± 1 SD

averaged 45.6 µM and the sum of N in biomass averaged 8 µM. Microbial abundances in controls did not show the marked increases or oscillations observed in the Delaware River water.

Experiment II

Initial DON concentrations were 46.5 and 33.5 µM in the Delaware and Hudson River water, respectively (Table 1). Average DON concentrations decreased by 32 and 17% by Day 4 in the Delaware and Hudson

Table 1. Rates of degradation of Delaware and Hudson River DON by estuarine microorganisms

DON source	Expt	Incubation period (d)	Avg initial DON (µM)	Fraction consumed[a] (%)	Avg rate[b] of utilization (µmol N d^{-1})
Delaware River	I	1–4	12.9	63	2.7
		4–15	4.7	72	0.1
	II	1–4	46.5	32	4.9
		4–8	31.6	40	0.9
Hudson River	II	1–4	33.5	17	1.9
		4–10	27.8	40	1.3

[a]Fraction of Day 1 concentration consumed
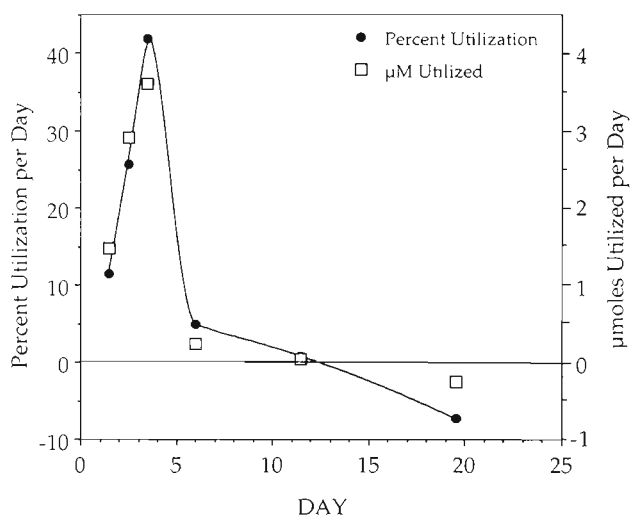[b]Rate of utilization during indicated incubation period

Table 2. Production of estuarine bacteria in waters originating from the Delaware and Hudson Rivers. Rates were calculated during the initial 2 d period of logarithmic growth. Water from the Hudson River was collected from an area where the anthropogenic input was primarily due to agricultural runoff, while that from the Delaware was collected downstream from wastewater treatment facilities. Control was deionized water. All water was filtered and brought to a salinity of 15 ppt prior to addition of bacteria (see 'Methods')

Site	Gross production (cells × 10^5 ml^{-1} d^{-1})	Specific production (d^{-1})	Population doubling time (d)
Control	0.5	0.1	5.8
Delaware River			
Expt I	20.7	0.74	0.9
Expt II	8.4	1.07	0.6
Hudson River			
Expt II	26.3	1.85	0.4

Hudson treatments (Table 3). PN concentrations increased by 11.1 µM, from 0.5 to 11.6 µM PN, in the Delaware treatment (particulate filters from A and B flasks analyzed together) (Table 3). In the Hudson treatment, PN increased from 0.9 µM to 14.4 (A replicate) and 8.8 µM (B replicate), or by 13.5 µM and 7.9 µM, respectively. DIP concentrations remained constant (Delaware 1 µM; Hudson 0.4 µM) throughout the experiment (data not shown). DON concentrations in control water remained constant (~4 µM) throughout the experiment, demonstrating, as for Expt I, that DON contained in the bacterial concentrate added at the beginning of the experiment was minimal and could not account for the pattern of DON decrease observed in the river water.

There was a rapid increase in the abundance and biomass of the estuarine bacteria growing on dissolved organics from the Delaware and Hudson Rivers (Fig. 4B, Table 2). The bacterial growth rate during the first 2 d on Delaware River DOM was 8.4 × 10^5 cells ml^{-1} d^{-1} and on Hudson River DOM was 26.3 × 10^5 cells ml^{-1} d^{-1} (Table 2). The maximum bacterial abundances reached in this experiment were similar to the maximum

River water, respectively, with a maximum decrease of 40% of the initial concentration over the course of the experiment for both treatments (Fig. 4A; Table 1). Rates of DON utilization in the Delaware treatment were higher during the first 4 d (15% d^{-1} or 4.9 µmol DON-N d^{-1}) compared to Days 4–8 (0.9 µmol DON-N d^{-1}) (Table 1). However, changes in DON utilization rates over the time course of the experiment were not as evident for the Hudson treatment (6.5% d^{-1} or 1.9 µmol DON-N d^{-1} during Days 1 to 4 and 1.3 µmol DON-N d^{-1} during Days 4 to 10). The fraction of biologically available DON in the Hudson River water may have been greater than 40% given that DON utilization continued at a relatively constant rate throughout the 10 d experiment. DIN concentrations in the Delaware and Hudson River water were 18 and 6.5 µM for ammonia, respectively, and 90 and 32 µM for nitrate plus nitrite, respectively. Throughout the experiments the DIN concentration increased by an average of 5 µM in the Delaware and decreased by 3 µM in the

Fig. 3. Expt I. Rates of utilization of DON in Delaware River water, expressed as percent of DON utilized per day and µmol DON-N utilized per day

densities in Expt I. However, average bacterial biovolume increased over time in Expt II, which resulted in an average community biomass that was 5 times that determined at the end of Expt I. Heterotrophic flagellates were not observed in Expt II, indicating that efforts to eliminate these predators were successful. At the end of the experiment, bacterial C calculated from biovolume was 62.5 and 66.1 µM in the Delaware and Hudson, respectively. The corresponding bacterial N averaged 12.5 µM in the Delaware and 13.3 µM in the Hudson River water (Table 3).

DISCUSSION

Mass balance of biomass production and dissolved nitrogen

In this study, concentrated assemblages of natural estuarine bacteria were added to sterile-filtered river water, which left riverine DOM as the only organic compounds for growth. A rapid increase in bacterial abundances in waters originating from both the Delaware and Hudson Rivers was mirrored by a decrease in DON concentrations. As detailed below, the DON concentrations decreased by 40 to 72% during the 10 to 15 d time course of the experiments, and were accounted for by increased microbial biomass plus mineralized DIN.

The bacterial production estimates calculated from increases in abundances during the first 2 d of these experiments (Table 2) are in general agreement with measurements of production in several estuarine systems (Table 4). The changes in microbial abundance during Expt I (Fig. 2), where the initial large increase in bacterial numbers was followed by a rapid increase in HNAN, also reflect the well-known ability of protists to reduce bacterial population levels (e.g. Sanders et al. 1992). The HNAN bacterivory reduced the abundance of bacteria and a series of damped predator-prey oscillations followed. While our estimates are not intended to precisely predict the actual population dynamics in the Delaware and Hudson estuaries, the bacterial growth rates on DOM and the predation by HNAN in these experiments fell within the range of previous observations and were ecologically reasonable.

The mass balances calculated below for changes in dissolved and particulate nitrogen in the water ignored any wall growth during the experiment. This was justified because growth of bacteria on the walls of the vessels was minor during the first 10 d of both experiments. Numbers of bacteria per cm² that were attached to the flasks were estimated from the bacteria attached to suspended glass cover slips. Using maximum observed densities of attached bacteria, which overes-
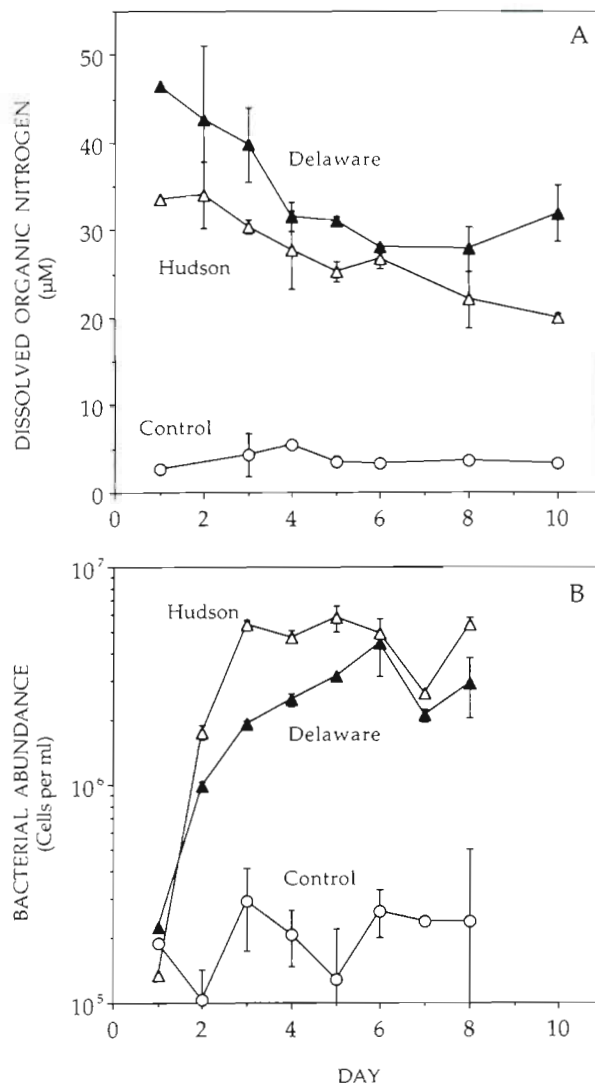

Fig. 4. Expt II. Changes in (A) concentration of dissolved organic nitrogen and (B) bacterial abundance in water originating from the Delaware and Hudson Rivers (▲ and △, respectively) and in controls (○). Means of 2 replicate chambers ± 1 SD

timates their relative importance early in the experiments, only 1.3% of the bacteria were attached in Expt I, and 4 to 6% in Expt II. The insignificant bacterial wall growth during the initial 10 d in our experiments agreed with the findings of Hagström et al. (1984). Biovolume estimates were made in Expt II for attached bacteria, and showed that they were smaller than the bacteria suspended in the water. Thus, attached bacterial biomass in these experiments did not exceed 4% of the total bacterial biomass during the period of rapid DON disappearance. Attached HNAN and large spirilla were observed on suspended coverslips after Day 10 in Expt I. This may account for a proportion of the observed, although small, decrease in DON between Days 10 and 15.

Table 3. Net utilization of dissolved organic nitrogen (DON), net production or utilization of dissolved inorganic nitrogen (DIN), and net production of particulate N (PN) in Delaware and Hudson River water (salinity 15 ppt) by estuarine bacteria during Expt II. Negative DIN numbers reflect decreases in DIN concentration, indicating bacterial utilization of DIN. Calculated DON utilization is the sum of increases in PN (estimated from either CHN analyses or bacterial biovolume) plus net change in DIN. Units: µM of N

	Delaware River			Hudson River		
Replicate:	A	B	Avg (±SD)	A	B	Avg (±SD)
Bacterial biomass production						
Particulate N analysis	11.1[a]	11.1[a]	11.1[a]	13.5	7.9	10.7 (3.9)
Calculated bacterial biomass	11.6	13.4	12.5 (1.3)	14.1	12.4	13.3 (1.2)
DIN mineralization or utilization	6	4	5 (1.4)	−2.9	−3.7	−3.3 (0.6)
DON utilization						
PN + DIN changes (range)	17.1–17.6	15.1–17.4	16.1–17.5	10.6–11.2	4.2–8.7	7.4–10
Measured DON decrease	17	20	18.5 (2.1)	13.2	13.9	13.5 (0.5)

[a]Average of A and B flasks. Water from the replicate flasks was combined for CHN analysis

Microbial utilization of DON during Expt I was estimated by 2 independent methods: (1) decreases in DON concentrations, and (2) calculations of N in bacteria and HNAN from biovolumes and abundances, plus changes in DIN concentrations. Maximum N biomass accumulation in bacteria plus HNAN was approximately 8 µM N and occurred on Day 7. There were no net changes in DIN concentration in Expt I. The increase in estimated microbial biomass is consistent with measured decreases in DON concentration (9.3 µM).

In Expt II, microbial utilization of DON was estimated by 3 independent methods: (1) decreases in DON concentrations, (2) chemical analysis of PN on filters, and (3) calculation of N in bacterial biomass based on bacterial biovolumes and abundances. Net changes in DIN were added to bacterial biomass accumulation to calculate total DON utilization rates for approaches 2 and 3. The 3 independent methods produced remarkably similar results (Table 3). In the Delaware River treatment, there was a 17 to 20 µM decrease in DON concentration during the course of the experiment (Table 3). The decrease in DON was matched by an increase of between 11.1 and 13.4 µM in particulate N (range of both methods) plus an increase of 4 to 6 µM DIN, for an average increase in PN plus DIN of 16.1 to 17.5 µM. The close agreement between the DON decrease (18.5 ± 2.1 µM) and the increase in PN plus DIN (16.1 to 17.5 µM) strongly suggests that most of the DON was incorporated into bacterial biomass, with a smaller amount of N mineralized as DIN. In the Hudson River treatment the decrease in DON (by 13.2 and 13.9 µM) was similar, although somewhat less than, the increase in particulate N minus utilization of ~3 µM DIN.

Table 4. Bacterial production estimates from several estuarine and riverine systems

Site	Gross production (cells $\times 10^5$ ml^{-1} d^{-1})	Specific production (d^{-1})	Reference
Danube River	47–78[a]	0.1–1.7	Berger et al. (1995)
Delaware estuary, USA	<1–60[b]	–	Coffin & Sharp (1987)
	–	<0.1–1.3	Hoch & Kirchman (1993)
Essex estuary, MA, USA	25–50	0.4–1.1	Wright & Coffin (1984)
Hudson River, USA	88	–	Findlay et al. (1991)
	8–20	–	Vaque et al. (1992)
Hudson estuary plume, USA	–	1.2–1.5	Ducklow & Kirchman (1983)
Meuse River, Belgium	–	0.3–0.7	Servais et al. (1985)
Rhône River plume, France	–	<0.1–0.3	Kirchman et al. (1989)
Seine River, France	–	0.2–4.3	Servais & Garnier (1993)
York estuary, VA, USA	7–75[a]	0.2–1.1	Ducklow (1982)

[a]Assumes 10^{-14} g C cell^{-1} [b]One out of 36 seasonal samples approached a gross productivity of 110×10^5 cells ml^{-1} d^{-1}

Bacterial incorporation of DON versus mineralization to ammonia

A high C:N ratio in DOM relative to the composition of bacteria should result in the conservation of incorporated N as biomass (i.e. there would be little release of DIN to the surrounding water) (Goldman et al. 1987, Gardner et al. 1996). C:N ratios of DOM in the Hudson River generally range from 15 to 50 (atoms) (Seitzinger 1995). Given that the C:N ratio of bacteria is often ~5:1 by atoms (Bratbak 1985, Goldman et al. 1987, Lee & Fuhrman 1987), one would predict little release of N as DIN in the Hudson River treatment, assuming that the C:N ratio of the DOM was similar to that reported previously for the Hudson River. In fact, the high C:N ratios of DOM in the Hudson, relative to bacterial biomass, would suggest that bacteria growing on this material would need an additional N source (e.g. ammonia) (Gardner et al. 1996). The results from the Hudson River treatment are consistent with the above prediction. There was no net mineralization of DON to DIN, but rather a net uptake of ammonia (3 µM) (Table 3). The C:N ratio of the DOM actually used by the bacteria is unknown because the pool of DOM likely includes many compounds which differ in their biological availability to bacteria. However, the lack of net mineralization of DON to DIN by the bacteria in the Hudson water is consistent with the high C:N ratio of the bulk DOM.

The C:N ratios of DOM in the Delaware River are lower than those in the Hudson, and generally range between 5 and 12 during spring (Culberson et al. 1987, Seitzinger & Sanders unpubl. data). There was no net production or utilization of DIN in Expt I. During Expt II, approximately 27 % of the DON utilized in the Delaware River water was mineralized as DIN (Table 3). The results from these 2 experiments are consistent with the C:N ratio of DOM in the Delaware River and indicate that the DOM utilized had a C:N ratio similar to, or was somewhat enriched in N relative to, the bacteria.

Additional studies would be needed in order to adequately evaluate the magnitude of various sources of DON (both allochthonous and autochthonous) to these rivers, including the C:N ratio of each source. However, the generally lower C:N ratios of DOM in the Delaware relative to the Hudson River may reflect the numerous point sources of organic matter (e.g. sewage treatment plants in the metropolitan Philadelphia area) that discharge to the Delaware River just upstream of the salinity intrusion zone. In contrast, in the Hudson River there are not major point sources in the region near the salinity intrusion area; DOM inputs are likely dominated by runoff from agricultural and forested areas and groundwater inputs along the length of the

Hudson (Howarth et al. 1991, Clark et al. 1992). A number of factors likely constrain bacterial growth, including the C:N ratio of DOM (Vallino et al. 1996). Despite different sources of DOM to these rivers, a similar percent (approximately 40 %) of the DON from both rivers was utilized within the 10 d time course of Expt II, and accumulation of PN was similar in both the Hudson and Delaware treatments. Additional measurements would be needed to confirm similarities during other seasons.

In both Expts I and II bacterial biomass stabilized after an initial period of rapid growth. This is a common pattern in batch cultures and indicates some growth limiting factor. There were ample amounts of DIN and DIP throughout both experiments, indicating that neither N or P was limiting. We suggest that carbon became limiting for bacterial biomass production, as the more labile carbon was utilized first, leaving the more refractory carbon. However, we cannot rule out the possibility that some micronutrient (e.g. Fe) became limiting.

Contribution of DON to estuarine eutrophication

The emphasis of most studies of N loading and its effect on estuarine processes has been on inputs of DIN, but DIN accounts for only a portion of the total N inputs. Twenty to over 90 % of the N inputs enter in the organic form (dissolved plus particulate), with 20 to 50 % often as DON. This is illustrated by the N composition of rivers which are the major conduit for N inputs to estuaries (Table 5). While riverine DOM may increase bacterial production in coastal waters (Chin-Leo & Benner 1992), the possible contribution of the inputs of riverine organic N to overall coastal eutrophication is currently unknown.

DOC mixing curves in some estuaries traditionally have been used to suggest that river inputs of DON are refractory (Montoura & Woodward 1983, Cadée 1984). However, interpretation of the mixing curves is subject to consideration of all DOM sources. In addition to the external inputs from rivers, the atmosphere, point and non-point sources, DON is produced within estuaries in the sediment and by planktonic organisms (e.g. Nixon & Pilson 1983, Bronk & Glibert 1991, 1993a, Nagata & Kirchman 1991). A DON budget for Narragansett Bay suggests that internal production of DON (105×10^6 mol N yr^{-1}; calculated from data in Nixon & Pilson 1983) is of a similar magnitude to external inputs (150×10^6 mol N yr^{-1}; Nixon et al. 1995). If the relative magnitude of internal production and external inputs is similar for other estuaries, then DON mixing curves would show conservative behavior if all of the internally produced DON was rapidly utilized (and none of

Table 5. Percent composition of inorganic and organic N in various rivers

River	% DIN	% DON	% PN	% ON	Reference
Mullica, NJ, USA	30	61	9	70	Durand (1988)
Mississippi, USA	53[a]			43	Turner & Rabalais (1991)
Amazon	25	29	46	75	Richey et al. (1991)
Mackenzie, Canada	10	15	75	90	Telang et al. (1991)
Unnamed stream, NH, USA	39	51	10	61	Likens (1985)
Blackstone, RI, USA	73	23	4	27	Nixon et al. (1995)
Pocono Mountain Stream, PA, USA	4			96	Seitzinger (unpubl.data)
Delaware, PA, USA	74	20	5	25	Culberson et al. (1987)
Rhode, MD, USA	30	14	56	70	Peterjohn & Correll (1984)
Como, CO, USA	10	90		90[b]	Meybeck (1982)
Lindaret, France	78	22		22[b]	Meybeck (1982)
Brevon, France	77	23		23[b]	Meybeck (1982)
Danube, Rumania	55	45		45[b]	Meybeck (1982)
Aare, Switzerland	73	27		27[b]	Meybeck (1982)
Reuss, Switzerland	56	44		44[b]	Meybeck (1982)
Rhine, Switzerland	74	26		26[b]	Meybeck (1982)
Rhône, Switzerland	56	44		44[b]	Meybeck (1982)
Ticino, Switzerland	57	43		43[b]	Meybeck (1982)
Missouri, USA	46	54		54[b]	Meybeck (1982)
Windrush, England	60			40	Heathwaite (1993)
Eastern Notec, Poland	29			71	Taylor et al. (1986)
Wda, Poland	70			30	Taylor et al. (1986)
Kullarna, Sweden	25			75	Lepistö et al. (1995)
Däntersta, Sweden	22			78	Lepistö et al. (1995)
Myllypuro, Finland	6			94	Lepistö et al. (1995)
Pahkaoja, Finland	21			79	Lepistö et al. (1995)
Avg world rivers	33			67	Meybeck (1982)

[a]NO_3+NO_2 only. [b]Minimum estimates as based on DON only; no PN data

the externally supplied DON was used), or if the amount of external DON utilized matched (spatially and temporally) the amount of internally produced DON that accumulated.

There is evidence that both internal and external sources of DON are biologically available in estuaries. Rapid utilization of DON released by phytoplankton in estuaries has been demonstrated (Bronk & Glibert 1993b), and it is known that bacteria and some phytoplankton can use low molecular weight DON produced in estuaries (e.g. dissolved combined and free amino acids, urea) (Paul 1983, Keil & Kirchman 1991, Middleboe et al. 1995). Few studies have directly examined utilization of external inputs of DON to estuaries. Experiments with riverine humic substances suggest that estuarine bacteria may use some portion (30 to 60%) of humic N, with subsequent regeneration of DIN contributing to increased phytoplankton production (Carlsson et al. 1993, 1995). However, actual decreases in humic N or increases in PN were not quantified in those experiments. In addition to bacterial degradation, a portion of DON may be photochemically degraded to ammonia; the ammonia would then be available for bacteria or phytoplankton production.

Approximately 20% of humic N in rivers of the southeastern U.S. may be photochemically degraded to ammonia in coastal ecosystems (Bushaw et al. 1996).

In the current study, natural assemblages of estuarine bacteria rapidly degraded a major portion of the total DON from 2 rivers, and mineralized or incorporated it into biomass. Rates of utilization of Delaware River DON ranged from 11 to 42% d^{-1} during the first 4 d, and then decreased to less than 5% d^{-1}. Hudson River DON was utilized at 6.5% d^{-1} during the first 4 d, and then decreased to less than 4% d^{-1}. The DON utilization rates may be conservative because they do not account for potential internal production of DON (i.e. release of DON by microorganisms).

There was a net utilization of between 40 and 72% of the DON in Delaware River water (Expts I and II) and 40% of the DON in Hudson River water (Expt II) within 10 to 15 d. The average freshwater residence time in New York Bay is 3 d and in Delaware Bay is approximately 80 d (Sharp et al. 1982), which encompasses the range of water residence times of many estuaries (Nixon et al. 1996). If the results of our study can be extrapolated to other systems, they suggest that in estuaries with residence times on the order of weeks to

months, such as Delaware Bay, river inputs of DON are first utilized primarily within the estuary. In contrast, in estuaries with residence times of less than a week, such as New York Bay, approximately half of the biologically available DON may be utilized within the estuary, with the remainder exported and utilized in continental shelf waters, such as in the Hudson River plume and New York Bight. River DON-N inputs that are incorporated into the biological cycle in estuaries, and that do not become subsequently denitrified or buried in the sediments, are also exported to shelf ecosystems.

The fraction of DON utilized in the Delaware and Hudson river experiments is similar to the fraction of DOC utilized (23 to 42%) in a phytoplankton bloom in the North Atlantic (Kirchman et al. 1991). However, both the rates of utilization and the fraction of the total DON utilized were greater for the Hudson and Delaware rivers than has been reported for DOC utilization in relatively unpolluted humic-rich blackwater rivers. For example, in a blackwater river in the southeastern USA, 14% of the total DOC was utilized during 3 d incubations (calculations based on data in Meyer et al. 1987). Three to 6% of the total DOC in 2 Amazon basin rivers was utilized during 3 to 4 d incubations (calculations based on data in Amon & Benner 1994). While relative rates of utilization of N and C on DOM may differ, it is likely that the differences in the portion of DON utilized in the Hudson and Delaware Rivers relative to the DOC utilized in the unpolluted blackwater rivers represent differences in overall DOM lability.

Nitrogen budgets for ecosystems are generally based on either DIN inputs (Nixon & Pilson 1983, Kempe et al. 1991, Alexander et al 1996) or total N inputs (Turner & Rabalais 1991, Boynton et al. 1995, Nixon et al. 1995, Michaels et al. 1996). The results of experiments presented in the current study demonstrate that DIN inputs underestimate, and TN inputs likely overestimate, the inputs of biologically available N inputs to estuaries. In order to develop a biologically available N budget for an ecosystem, DIN inputs, plus that portion of the organic N (both particulate and dissolved) that is biologically available, must be quantified. The degree to which organic N is available, and contributes to production and eutrophication may depend on the source of the organic matter. Clearly, development of such a budget for any ecosystem will require considerably more data on the biological availability of organic N sources than is currently available.

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