

# Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies

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**ABSTRACT:** The assimilation of dissolved inorganic N (DIN) by heterotrophic microorganisms is a potentially large source of organic N to aquatic ecosystems, particularly those that receive high loads of both terrestrial organic matter (with high C:N values) and DIN. We investigated this rarely studied process in such a system (the Hudson River Estuary, USA) using: (1) *in situ* incubations of terrestrially derived particulate organic matter; (2) laboratory microcosms with terrestrially derived dissolved organic matter; and (3) ecosystem budgets of bulk N and <sup>15</sup>N. We also analyzed the <sup>15</sup>N and <sup>13</sup>C content of primary producers and invertebrate and fish consumers to demonstrate how food web dynamics can be incorrectly interpreted if microbially assimilated DIN (MAD) is not considered. During 3 mo *in situ* incubations, %N content of terrestrial material increased by about 3-fold (C:N decreased from 70 to 25) and δ<sup>15</sup>N values increased from –4 to 9‰. Similarly, in microcosms (where δ<sup>15</sup>N of DIN was 10 000‰), δ<sup>15</sup>N of POM and DOM increased after 105 d to over 5000 and 1000‰, respectively. Finally, an ecosystem budget suggests that net MAD is up to 13 g N m<sup>–2</sup> yr<sup>–1</sup> which is 4-fold larger than net N assimilation by phytoplankton. Thus, both incubations and ecosystem budgets suggest that MAD is large in the Hudson. Traditional food web analyses based on <sup>15</sup>N and <sup>13</sup>C which ignored MAD would result in the conclusion that terrestrial organic matter was unimportant to consumers in the Hudson. When the large input of MAD is recognized, a likely interpretation becomes: terrestrial organic carbon is important to consumers but a large part of organic N originates from heterotrophic rather than autotrophic assimilation.

**KEY WORDS:** N assimilation · Microbial · <sup>15</sup>N · Estuary · Food web

## INTRODUCTION

Microbial biomass and detrital organic matter can be an important proximate food source to grazers in aquatic systems (Fig. 1; Findlay & Tenore 1982, Tenore 1988). While the organic carbon in these food sources is ultimately from photosynthesis (in both the aquatic system and its watershed), the organic nitrogen may have been produced either by autotrophic (AAD) or heterotrophic microbial (MAD) assimilation of dissolved inorganic nitrogen (DIN; Fig. 1). For N, therefore, heterotrophic microbes have 2 roles: the re-packaging or concentrating of autotrophically produced organic N and the actual production of new organic N

through MAD. Although there are studies that have clearly demonstrated the importance of microbes as a proximate source of organic N (Findlay & Tenore 1982), few have examined the source of N to microbes (MAD vs AAD). It is generally assumed, however, that AAD is the major source of organic N to a system and models of N cycling often completely ignore MAD (e.g. Mantoura et al. 1988, Boynton et al. 1995, Mcquire et al. 1995).

Although there are relatively few studies of MAD, it has been investigated in marshes, small woodland streams, and lakes (e.g. Howarth & Fisher 1976, Carpenter & Adams 1979, Meyer & Johnson 1983, White & Howes 1994). Additionally, there are a few studies on MAD in marine coastal waters (Zieman et al. 1984, Wheeler & Kirchman 1986, Horrigan et al. 1988). These studies in aquatic systems, along with many in

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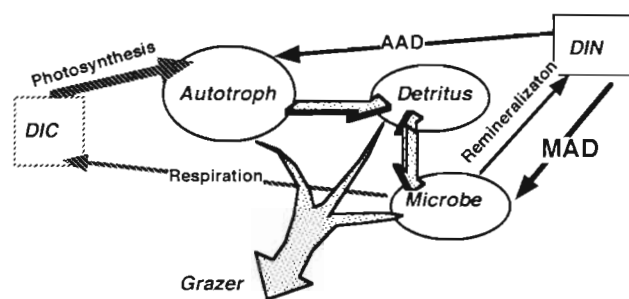


Fig. 1. Diagram of the flow of nitrogen and carbon from inorganic pools into autotrophs, detritus and microbes. Dissolved inorganic N (DIN) can enter the organic pool both through autotrophic assimilation of DIN (AAD) and microbial assimilation of DIN (MAD). Autotrophs include phytoplankton, macrophytes and terrestrial vegetation (allochthonous material to aquatic systems) and microbes include bacteria and fungi. It is possible for detritus to become enriched in N with respect to the autotrophically produced organic material both by conserving N (having low remineralization as compared to respiration) or by MAD

terrestrial systems (e.g. Suberkropp & Chauvet 1995, Stark & Hart 1997), often suggest that MAD may be influenced by the DIN concentration of the ambient environment such that MAD is likely to be elevated in high DIN systems. Further, studies indicate that MAD may be larger when material being processed has low initial N content (e.g. Zieman et al. 1984). Terrestrial material often has N content well below that of phytoplankton or macrophytes (Vitousek 1984, Hecky et al. 1993). Thus, MAD could be particularly important in aquatic systems with both large inputs of terrestrial material and high DIN concentrations.

For many rivers and oligohaline estuaries a major carbon input is terrestrially derived (allochthonous) material (Richey et al. 1978, Peterson et al. 1986, Allan 1995, Hopkinson & Vallino 1995, Welcomme 1995). In addition, these systems frequently have DIN concentrations in surface waters that exceed  $20 \mu\text{M}$  even during summer months (Fonselius 1978, Cifuentes et al. 1988, Cole et al. 1993, Caraco et al. 1997). By comparison, most estuaries and coastal waters have DIN concentrations below  $5 \mu\text{M}$  during much of the year (Corner & Davies 1971, Fonselius 1978, Oviatt et al. 1995). Thus, rivers and oligohaline estuaries are sites where MAD may be particularly important. The enrichment of allochthonous material by MAD in rivers and oligohaline sections of estuaries could have consequences for food quality of material delivered to more saline sections of estuaries and coastal waters.

In this study we examine MAD and several consequences of MAD in the fresh to oligohaline sections of a tidal river, the Hudson. This section of the Hudson has been extensively studied and much is known about dissolved nutrient dynamics, allochthonous car-

bon inputs and within-system primary and secondary production. We studied the importance of MAD in the tidal fresh/oligohaline Hudson River Estuary. This habitat extends nearly 200 km from near Albany, NY, USA (upstream) to about 50 km north of New York City (downstream). During summer this section of river is relatively warm ( $20$  to  $30^\circ\text{C}$ ) throughout the mixed water column (A. Lampman, N. F. Caraco & J. J. Cole unpubl.). Nutrient concentrations throughout this section of river are high (Caraco et al. 1997, Lampman et al. unpubl.). For example, during summer 1996 at a mid-river station (where and when *in situ* incubations were done in our microcosm study; see below),  $\text{NO}_3$  and  $\text{NH}_4$  concentrations averaged  $40$  and  $3 \mu\text{M}$ , respectively, while  $\text{PO}_4$  concentrations averaged  $1.1 \mu\text{M}$ . Further,  $\text{NO}_3$  concentrations remained above  $30 \mu\text{M}$  throughout this summer (Lampman et al. unpubl.).

Much is known about organic carbon inputs and heterotrophic use of these inputs in the tidal Hudson (Findlay et al. 1991, Cole et al. 1992, Pace et al. 1992, Howarth et al. 1996, Strayer et al. 1996). Based on watershed modelling, terrestrial inputs are large and average approximately  $650 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Howarth et al. 1996). In the same section of the Hudson autochthonous carbon inputs are relatively low due to extreme light limitation (Cole et al. 1992, Harley & Findlay 1994) and grazer limitation due to a zebra mussel invasion (Caraco et al. 1997). For example, for both phytoplankton and macrophytes the net production is presently approximately  $30 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Howarth et al. 1996, Caraco et al. 1997).

The tidal freshwater Hudson has a relatively long water residence time. Averaged over the year, residence time is approximately 1 mo and during the summer growing season residence time is closer to 3 mo (Limburg et al. 1986, Caraco et al. 1997). Thus, allochthonous organic matter inputs, entering primarily at the top of the river (Howarth et al. 1996, Swaney et al. 1996), remain in this region a relatively long time before being exported and, thus, are available for processing by heterotrophs within the system (Findlay et al. 1992). Consequently, bacterial production is high (ca  $200 \text{ g C m}^{-2} \text{ yr}^{-1}$ ) and exceeds net primary production in the Hudson by over 5-fold (Findlay et al. 1991, Cole et al. 1992, Caraco et al. 1997). This bacterial production would demand a carbon respiration of approximately  $400 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Findlay et al. 1992). Further, respiration by one benthic invertebrate, the zebra mussel, is equal to about  $50 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Strayer et al. 1996). Considering only the demands of planktonic bacteria and one invertebrate, then, the total heterotrophic carbon demand exceeds the autotrophic input by about  $400 \text{ g C m}^{-2} \text{ yr}^{-1}$ . In agreement with this, calculations of both net gas flux and diel gas cycling suggest that the Hudson is net heterotrophic, with dif-

ferent studies giving estimates that vary from about 100 to 500 g C m<sup>-2</sup> yr<sup>-1</sup> (Howarth et al. 1996, Raymond et al. 1997).

Although the exact value of heterotrophic processing of allochthonous material is uncertain, all data clearly suggest that the respiration of allochthonous material by heterotrophs is important in the Hudson. Further, autochthonous production by macrophytes and phytoplankton is rather small. Terrestrial material could, therefore, be an important food source for consumers in this system. The  $\delta^{15}\text{N}$  signal of juvenile herring species resident to the freshwater tidal Hudson is, however, about 15‰ higher than terrestrial material (Limburg 1998). If the organic N originated from assimilation by terrestrial autotrophs, it would require that herring in the Hudson were eating at trophic level 5 (Fry 1991, Michener & Schell 1994). This possibility seems unlikely as these fish are primarily planktivores. The results, therefore, indicate that the source of organic N for fish is substantially more  $^{15}\text{N}$  enriched than terrestrial material with a  $\delta^{15}\text{N}$  value of about -2‰. This  $^{15}\text{N}$  enriched source of organic N could potentially originate from MAD during decomposition of terrestrially derived material within the Hudson.

In this study we examine MAD during the microbial processing of allochthonous organic material. To look experimentally at this process, we used both *in situ* litter bag studies and laboratory microcosms. Both the  $^{15}\text{N}$  content and the bulk N content of decomposing material were followed during these incubations to trace microbial immobilization of DIN directly. Further, we used a bulk N and  $^{15}\text{N}$  budget to calculate MAD and compare it to AAD in the Hudson (Fig. 1). Lastly, we discuss how MAD can change the view, gained from food web analysis using multiple isotopes (Hamilton et al. 1982, Peterson et al. 1985, Deegan et al. 1990, Sullivan & Moncreiff 1990, Fry 1991, Wada et al. 1993, Angradi 1994), of the importance of allochthonous material as a food source.

## METHODS

**Experimental and sampling rationale.** We tried to evaluate MAD by several independent approaches. The litter bag experiments were designed to test if changes consistent with MAD occurred within the Hudson. These experiments suffer from the problem of clearly associating changes in chemistry of detrital material with MAD versus other mechanisms of change. The microcosm experiments were performed with a  $^{15}\text{N}$  enriched DIN source so that MAD could be more unequivocally demonstrated when terrestrial material was decomposed. The ecosystem budget was designed to see if there appeared to be an imbalance

in organic N budgets (or organic N isotope content) that was consistent with MAD. The ecosystem budget is free of experimental artifacts that may occur due to enclosure in small containers but is associated with relatively large errors.

One obvious implication of MAD is that it enriches terrestrial material in N and provides new organic N to aquatic systems. Less obviously, perhaps, MAD can alter dramatically interpretation of food web analysis based on the combination of  $^{13}\text{C}$  and  $^{15}\text{N}$  data (e.g. Hamilton et al. 1982, Peterson et al. 1985, Deegan et al. 1990, Sullivan & Moncreiff 1990, Fry 1991, Wada et al. 1993, Angradi 1994). To demonstrate this we use isotope data on a variety of trophic levels in the Hudson.

***In situ* litter bag experiment.** Leaves from red oaks *Quercus rubrum* were collected immediately after autumn leaf fall from the mid-Hudson region within the Hudson River watershed. The leaves were air dried and stored at room temperature. During early summer of 1996, 25 mesh bags (1.5 mm mesh) were filled with approximately 4 leaves/bag (1 to 2 g) and were sewn closed with nylon thread. Bags were deployed near a major channel in the Hudson River at river km 150 at a site that is 13 m deep (mean low water). Bags were deployed 3 m off the bottom and 10 m from the surface. Light levels at this depth averaged less than  $10^{-7}$   $\mu\text{mol}$  quanta m<sup>-2</sup> s<sup>-1</sup> at noon during the incubation period. These levels are orders of magnitude too low to sustain any photosynthesis in this system (Cole et al. 1992).

Leaves were collected after 17, 39, 80 and 115 d of incubation. Between 5 and 7 replicate bags were collected at each time point. Bags were immediately put into opaque coolers and transported to the laboratory. Debris and macroinvertebrates that had entered the bags (some amphipods) were thoroughly rinsed from leaves and leaves were dried at 40°C. Dried leaves were then pulverized in a plant mill and stored in a desiccator. Analysis of total C, N,  $^{15}\text{N}$  and  $^{13}\text{C}$  was done at the Isotope Laboratory of the University of Alaska at Fairbanks.

**Laboratory microcosms.** An extract of allochthonous material was prepared by adding 5 g of dried oak leaves to 1 l of nanopure water and heating to 95°C for 0.25 h. When this mixture had cooled, the liquid fraction was decanted off the top. This extract was then refrigerated for 1 d before being added to the microcosms.

The water for the incubations was collected from the Hudson River at km 150 on 1 December 1996. The water was filtered through a GF/D filter to remove most of the suspended matter. The filtered water was then placed in triplicate 11 l containers. Each container was spiked with 57 ml of the extract such that the DOC content of the Hudson was increased by 3 mg l<sup>-1</sup> (approximately doubling ambient DOC). 2.5 ml of

11 mM NaNO<sub>3</sub> (100% in <sup>15</sup>N, from Isotech) was added to each of the 11 containers. Nitrate was added as the DIN in the Hudson is primarily nitrate. This addition increased the NO<sub>3</sub> content of the water from 40 μM to 42.5 μM and increased the δ<sup>15</sup>N value of the NO<sub>3</sub> to approximately 10 000‰.

Immediately after <sup>15</sup>NO<sub>3</sub> and extract additions, 2 l samples were removed from each of the 3 replicate 11 l containers and processed (see below). These initial samples served as controls for any contamination of DON that may have occurred with the added DIN. The remaining 9 l samples were then placed in complete darkness and incubated at room temperature. Bottles were continuously aerated during incubations to ensure oxic conditions. After 7 and 35 d, 2 l samples were removed to be processed.

The 2 l samples were filtered through 2.5 cm GF/F filters to remove most of the particulate microbial biomass that had formed during the incubation. The large molecular weight DOM in the filtrate was then separated from the inorganic N with a Pelican reverse flow filtration system equipped with a 1000 NMW filter pack. In tests with DOM-NO<sub>3</sub> solutions we found that this pore size allowed all of the inorganic N to pass through the filtration unit but only 15% of added DOM was retained.

The retained DOM was concentrated from the original 2 l volume to a volume of 400 ml. We further concentrated this material by evaporation at 80°C to 5 ml. This evaporate was then pipetted in 0.2 ml aliquots onto 5 GF/F filters and dried at 40°C. The dried DOM and POM samples were stored in a desiccator until being sent for processing at the Marine Biological Laboratory Isotope Facility, Woods Hole, MA, USA or the Horn Point Environmental Laboratory, Cambridge, MD, USA.

**Samples of Hudson River food web.** Samples for isotopic analysis were collected in the Hudson River from a number of locations between km 245 and 50. Samples were primarily collected during the summer growing season in 1995 and 1996 (Table 1). Samples included: seston collected on GF/F filters; macrophytes; zooplankton; benthic invertebrates; and a variety of larval, juvenile and adult fishes of several species (Table 1). In all, over 200 samples were analyzed for <sup>15</sup>N and/or <sup>13</sup>C. Zooplankton and larval fish were collected with net hauls. Most of these samples were picked under a dissecting microscope to achieve pure samples. For some zooplankton, net samples were concentrated on filters and checked with the dissecting microscope to ensure that samples were pure. Benthic invertebrates were collected by SCUBA diver, from box core samples, or from net tows on night collected samples. Juvenile and adult fish were collected by seine or by hook and line. For larger adult fish, only filets were analyzed. For smaller fish, the entire fish was analyzed (Table 1).

All samples were dried and pulverized before being sent for analysis. The seston samples and the zooplankton and larval fish samples were analyzed at the Marine Biological Laboratory Isotope Facility. Most of the larger fish, macrophyte and invertebrate samples were analyzed at the Isotope Laboratory at the University of Alaska at Fairbanks.

**Ecosystem budget.** We tried to generate organic N budgets that balanced both bulk organic N and <sup>15</sup>N. To calculate bulk N, we relied heavily on the recently published organic carbon budget of Howarth et al. (1996) for the Hudson. We used values of Howarth et al. (1996) to calculate allochthonous load, macrophyte production, and respiration of allochthonous material

Table 1. Description of samples for isotope analysis from the Hudson River. We show, for each group we identify in the text, the isotope that was measured, the tissue or compound sampled (What), the number of samples analyzed (n), the range of locations sampled (km), the period of the year we sampled (Timing), and what taxa were included (Taxa)

	Group	Isotope	What	n	km	Timing	Taxa
Producers	Allochthonous	C+N	Leaves	4	130–150	October	<i>Quercus</i> , <i>Acer</i>
	Phytoplankton	C	DIC – 20	45	60–220	May–Sep	
	Phytoplankton	N	See text	3	60–150	Jun–Aug	<i>Enteromorpha</i> , <i>Microcystis</i>
	S. macrophytes	C+N	Leaves + stems	10	80–180	Jul–Aug	<i>Valisneria</i>
	E. macrophytes	C+N	Leaves + stems	7	150–180	Jul–Aug	<i>Trapa</i>
Organic pools	Seston	C+N	GF/F filter	15	65–150	Jun–Oct	
	Sediment	C+N	Upper 10 cm	7	100–200	Jun–Sep	
Consumers	Zooplankton	C+N	Entire	25	65–225	Jun–Aug	<i>Bosmina</i> + misc. copepods
	Predatory inv.	C+N	Entire	15	65–150	Jun	<i>Leptodora</i>
	Larval fish	C+N	Entire	14	65–150	Jun	<i>Anchoa</i> , <i>Alosa</i> , <i>Morone</i> , <i>Osmerus</i>
	Benthic inv.	C+N	Entire (no shells)	37	80–220	Jun–Oct	<i>Dreissena</i> , <i>Gammarus</i> , chironomids
	YOY and adult fish	C+N	Entire or fillet	31	48–200	Jun–Aug	<i>Anchoa</i> , <i>Alosa</i> , <i>Morone</i> , <i>Perca</i> , <i>Notropis</i> , <i>Ictalurus</i> , <i>Anguilla</i> , <i>Lepomis</i> , <i>Fundulus</i>



originating from sewage and terrestrial runoff. Phytoplankton production was based on recent estimates (Caraco et al. 1997) which take into account changes caused by the introduction of the zebra mussel (1992 to present).

To transform the organic C budget to an organic N budget, we used C:N values of 7, 15, and 10 for phytoplankton, macrophytes, and sewage, respectively (Mackenthum 1973, Hecky et al. 1993). The C:N of organic material originating with terrestrial material is critical to the budget, but calculations of this value could differ if it is assumed that terrestrial material enters primarily as leaf material or soil humic material. We used these 2 extremes in our calculation, where scenario 1 used our measured C:N in leaf material (70) and scenario 2 used a value of 30 which is the approximate C:N of soil humic material. Organic N leaving the Hudson is based on direct measurements of total N less measured DIN in the lower reaches of the river (Lampman et al. unpubl.).

We used Howarth et al.'s (1996) total system respiration and we assume that all inputs (net phytoplankton production, terrestrial load, etc.) had equal ratios of remineralization to export. Had we assumed that autochthonous production was respired proportionally more than terrestrial loads it would have resulted in higher estimates of MAD. We transformed C respiration into N remineralization by assuming that during decomposition C and N were remineralized at the same rates. This assumption is in agreement with comparative losses of 'original N' and C in experiments (in 'Discussion').

In the  $^{15}\text{N}$  budget,  $\delta^{15}\text{N}$  values of seston leaving the Hudson, sediment burial, and phytoplankton and macrophyte production are based on the average of our measurements.  $\delta^{15}\text{N}$  of sewage is based on measurements of Van Dover et al. (1992).  $\delta^{15}\text{N}$  of terrestrial input is based on our measurements of leaf material in scenario 1. For scenario 2, we used a  $\delta^{15}\text{N}$  value of 4‰; this value is on the heavy side for soil organic matter (Nadelhoffer & Fry 1994).

## RESULTS

### *In situ* litter bag experiments

There was rapid loss of material from the litter bags. Total mass of material decreased to 50% of the initial mass during the first month and by Day 115 was near 25% of initial mass (Fig. 2a). Not surprisingly, total carbon showed a similar pattern of loss as did total mass but had a slightly higher loss rate than did mass as a whole (Fig. 2a). The total mass of N in the litter bags showed a somewhat different pattern than

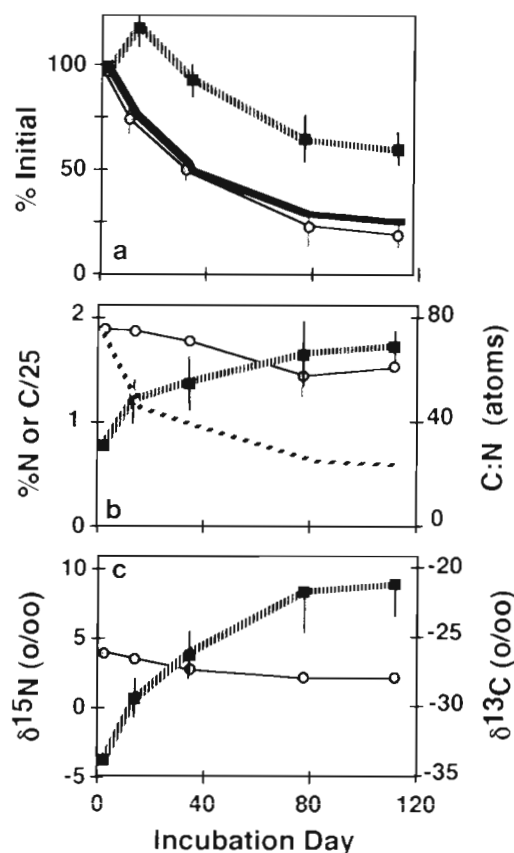


Fig. 2. Results of the *in situ* 120 d litter bag experiment in the Hudson River. (a) Changes in mass of material as expressed as percent of that initially in the bag. Included are: N mass (stippled line and  $\blacksquare$ ); total weight (heavy solid line); and C mass (O). Standard deviations (SD) are shown for N and C. (b) Change in composition of the material remaining in the bag. Included are %N ( $\blacksquare$ ) and %C (O), where '%' means % of dry weight. Also shown is the C:N ratio of the material (dashed line). SDs are shown for %N and %C. Note that %C is divided by 25. (c) Isotope content of remaining material. Shown are:  $\delta^{13}\text{C}$  (O) and  $\delta^{15}\text{N}$  content ( $\blacksquare$ ). SDs are shown for both N and C although they are too small to be seen for C.

weight or carbon loss. Amount of N in the litterbags actually increased to near 120% of initial values before decreasing to about 60% of that initially there by Day 115.

The N content of oak leaves changed dramatically over the incubation. Initially leaf material had low N content. N averaged 0.8% of dry weight and the C:N was near 70 (atom based; Fig. 2b). After only 17 d of *in situ* incubation, the N content increased to 1.2% of dry weight and by the end of the experiment the N content of material had increased to 2% and C:N ratios had declined to near 25:1 (by atoms). Unlike the large changes in N content, carbon content of material was quite constant with only a slight decrease from the initial 50% of dry weight.

Like the bulk N content, the  $\delta^{15}\text{N}$  of N in leaves also changed greatly over the incubation (Fig. 2c). Initially the  $\delta^{15}\text{N}$  value of the litter bag material (oak leaves) was  $-4\text{‰}$ . By Day 17,  $\delta^{15}\text{N}$  values had increased to  $0\text{‰}$  and by Day 115 the value was near  $10\text{‰}$ . The  $\delta^{13}\text{C}$  content of leaf material also changed during the *in situ* incubation (Fig. 2c). The  $\delta^{13}\text{C}$  started off at  $-27\text{‰}$  and steadily decreased to  $-28.5\text{‰}$  by Day 115. This decrease is consistent with preferential lignin retention during decomposition (Benner et al. 1987). Whatever the cause, however, by comparison to the  $\delta^{15}\text{N}$  change, change in  $\delta^{13}\text{C}$  value over the incubation was small.

### Microcosm experiments

In complete darkness,  $^{15}\text{N}$  content of organic N increased dramatically when the surrounding DIN was enriched in  $^{15}\text{N}$ . Initially, the DOM material retained by the Pelican filter had a  $\delta^{15}\text{N}$  value of  $11 \pm 4.5\text{‰}$  (Fig. 3). After 1 wk this same DOM fraction had a  $\delta^{15}\text{N}$  value of  $673 \pm 200\text{‰}$  (Fig. 3) and by 105 d had increased to  $1229 \pm 290\text{‰}$ .

Initially, essentially no particulate organic material was present in this experiment (only a small microbial inoculum). After 7 d substantial POM was formed and epifluorescent microscopic analysis suggested this particulate matter was composed in large part of a combination of fungi, actinomycetes, and small bacteria but appeared to contain some flocculent material that was possibly formed abiotically from DOM during bubbling in the experiment. This POM formed during the experiment was highly enriched in  $^{15}\text{N}$ . At Days 7 and 105 this POM fraction had a  $\delta^{15}\text{N}$  value of  $3556 \pm 840$  and  $5157 \pm 1358\text{‰}$ , respectively (Fig. 3).

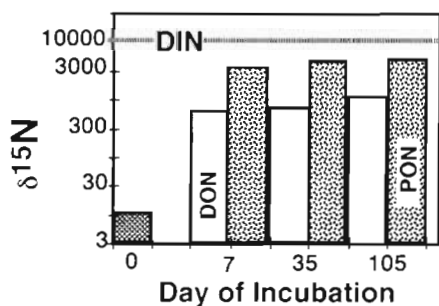


Fig. 3. Results from the microcosm study of microbial assimilation of DIN (MAD). Shown are  $^{15}\text{N}$  content of DOM (light stippling) and POM (heavy stippling) after 0, 7, 35, and 105 d of incubation in DIN of high  $^{15}\text{N}$  content (10000 ppt, shown by dashed line). Values at Day 0 are initial  $^{15}\text{N}$  of dissolved organic material (a mixture of leaf extract and Hudson DON, see text). The large increase in  $^{15}\text{N}$  content of POM and DOM (note log scale) indicates substantial MAD and subsequent leakage of this material into the DOM material

### Hudson River food web

The  $^{13}\text{C}$  of autotrophic C sources to the Hudson varied widely and allochthonous inputs fell within the range of autotrophic sources (Fig. 4). All autotrophs, on the other hand, had relatively similar and heavy  $\delta^{15}\text{N}$  values while terrestrial material had very different and far lighter  $\delta^{15}\text{N}$  signals (Fig. 4).

Terrestrial leaf material collected from maples (*Acer*) and oaks (*Quercus*) in the Hudson watershed had average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-27$  and  $-2\text{‰}$ , respectively. Submerged macrophytes in the Hudson had average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-22$  and  $+8\text{‰}$ , respectively, while emergent macrophytes had similar  $^{15}\text{N}$  values, but higher  $\delta^{13}\text{C}$  values ( $-26\text{‰}$ ).

Both the  $^{13}\text{C}$  and  $^{15}\text{N}$  content of phytoplankton and in the Hudson are based on indirect measures.  $\delta^{13}\text{C}$  is

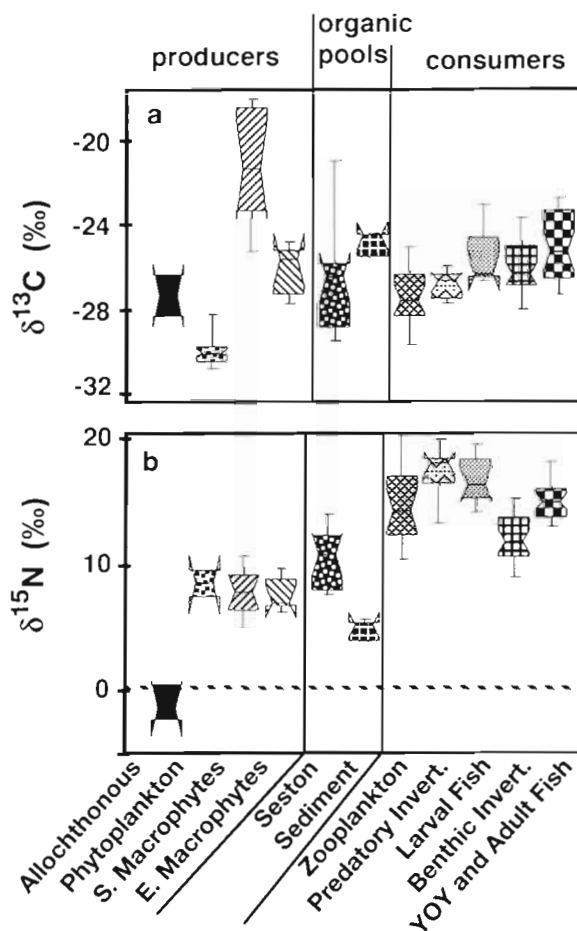


Fig. 4. Box-whisker plot of (a)  $^{13}\text{C}$  and (b)  $^{15}\text{N}$  (lower panel) content of primary producers, detrital organic pools and primary and secondary consumers (see Table 1). Central lines represent mean values, boxes and lines encompass 75 and 90% of data, respectively; notches represent 95% confidence interval on median value

based on an assumed  $-20\text{‰}$  depletion as compared to the DIC pool ( $-10 \pm 1\text{‰}$ ; Cole unpubl.) and is, thus, estimated as  $-30\text{‰}$ . The  $\delta^{15}\text{N}$  value for phytoplankton was inferred from the average of  $\delta^{15}\text{N}$  of a non rooted macroalga (*Enteromorpha* sp.), attached filamentous algae, and  $\delta^{15}\text{N}$  of seston during times of high phytoplankton abundance (during summer 1991 prior to zebra mussel invasion; Caraco et al. 1997). All of these 'phytoplankton surrogates' had  $\delta^{15}\text{N}$  values close to those of emergent and submergent macrophytes

( $+8\text{‰}$ ; Fig. 4). As phytoplankton have a discrimination of near  $-7\text{‰}$  as compared to inorganic N pools (Goerick et al. 1994), these  $^{15}\text{N}$  values imply a DIN pool of near  $15\text{‰}$ .

Despite a relatively wide range in  $\delta^{13}\text{C}$  values of C sources (ca  $8\text{‰}$ ), all of the consumers and the organic pools had  $^{13}\text{C}$  values that fell in a relatively narrow range (Fig. 4). The range in average  $\delta^{13}\text{C}$  values for these groups was less than  $2\text{‰}$ , with zooplankton having the lowest average value ( $-27.5\text{‰}$ ) and fish having the highest average value ( $-25\text{‰}$ ). The variation in  $^{13}\text{C}$  content was relatively large within some groups as they contained a mixed group of organisms. For example, fish included planktivores, piscivores, and benthic feeders. For seston, the high variation was seasonally related. Fall samples (during macrophyte die-off; Findlay pers. comm.) were enriched (less negative  $\delta^{13}\text{C}$  values) in  $^{13}\text{C}$  (like submergent macrophytes), while spring-summer samples were relatively light.

The  $\delta^{15}\text{N}$  values of consumers and seston were high as compared to all primary producers, and were particularly high as compared to allochthonous material ( $\delta^{15}\text{N}$  of  $-2\text{‰}$ ; Fig. 4). The  $\delta^{15}\text{N}$  of seston was on average  $12\text{‰}$  higher than this allochthonous material and consumers were from  $14$  to  $19\text{‰}$  higher than allochthonous material. The high values for consumers suggest they were consuming primarily material with high  $\delta^{15}\text{N}$  values. For example, primary consumers (zooplankton and benthic invertebrates) had average  $\delta^{15}\text{N}$  values of  $12$  and  $14\text{‰}$ , respectively. This suggests that these consumers were feeding on material with average  $\delta^{15}\text{N}$  values of near  $9$  and  $11\text{‰}$ , respectively.

As with  $\delta^{13}\text{C}$  values, some of the variation in  $\delta^{15}\text{N}$  values within groups was due to mixing of different types of organisms within a group and seasonal variation in samples. Additionally, however, some of the variation in  $\delta^{15}\text{N}$  is also due to spatial variation (Fig. 5). That is, the  $\delta^{15}\text{N}$  values increased significantly down river for seston, larval fish, predatory invertebrates, and benthic organisms (Fig. 5). Further, linear regression fits of  $\delta^{15}\text{N}$  versus river km for all of these groups showed similar slopes ( $0.04\text{‰ km}^{-1}$ ) that suggested an  $8\text{‰}$  increase in  $\delta^{15}\text{N}$  value over the  $200\text{ km}$  study reach of the Hudson River Estuary.

### Ecosystem budget

The organic N budget of the Hudson suggests that inputs of organic N are less than outputs if MAD is not considered. Inputs, including net production by phytoplankton, macrophytes, and sewage loading, contribute  $3$ ,  $2$  and  $6\text{ g N m}^{-2}\text{ yr}^{-1}$ , respectively. The terrestrial input of N varies with assumption about the origin of terrestrial material (scenario 1 = leaf material origin

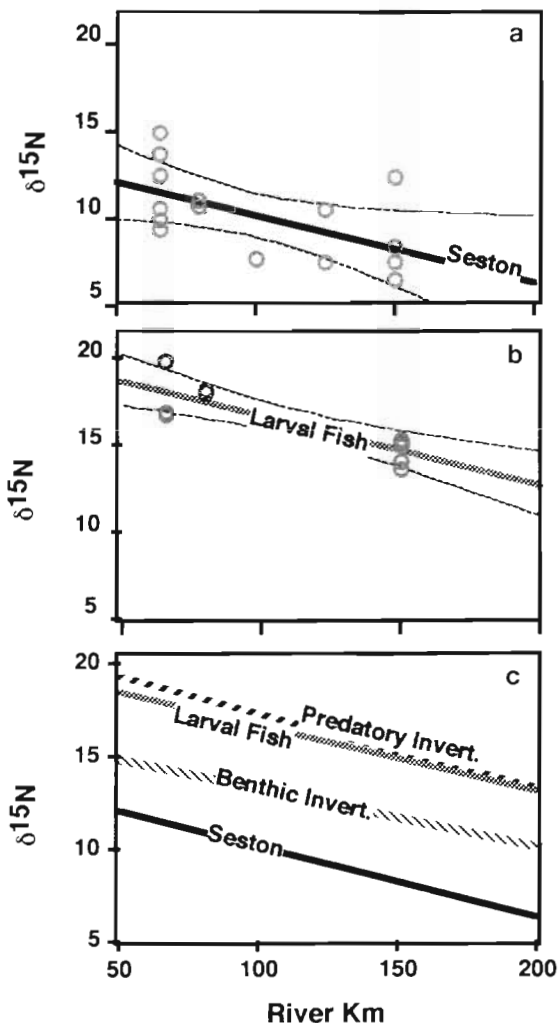


Fig. 5. Longitudinal variation in  $^{15}\text{N}$  content of seston and several groups of consumers in the Hudson River. (a)  $\delta^{15}\text{N}$  values (in  $\text{‰}$ ) of seston in the river. (b)  $\delta^{15}\text{N}$  values for larval fish. In these 2 panels data points, linear regression lines, and 95% confidence intervals around regression lines are shown. In (c) we show linear regression lines only for all groups that showed a significant ( $p < 0.05$ ) trend of  $^{15}\text{N}$  content with river km. For all of these groups the slopes are not significantly different from each other. Note that km 50 is the downstream-most reach of the study, and the intercept at km 50, therefore, represents the  $^{15}\text{N}$  content of materials leaving the system

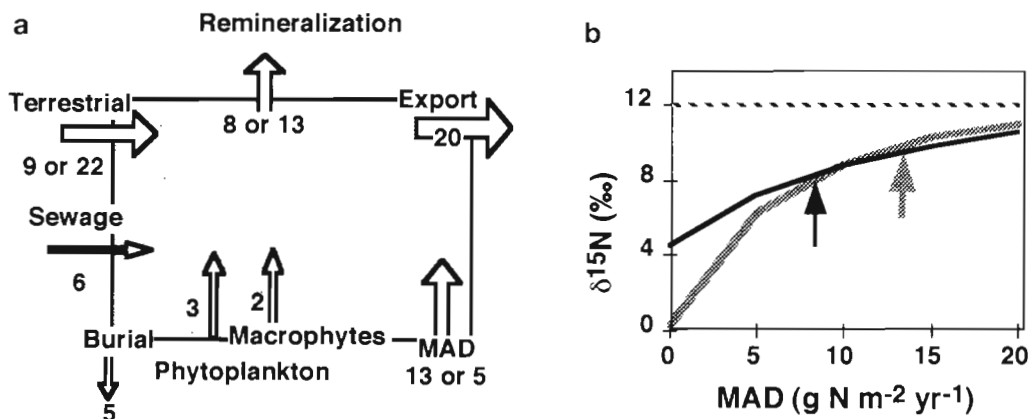


Fig. 6. (a) Bulk N budget for the Hudson River estuary (km 250 to 50) for 2 scenarios of terrestrial loading. Scenario 1 assumes terrestrial material to be primarily plant leaf material, scenario 2 assumes highly altered soil organic material with higher N and  $^{15}\text{N}$  content (see text). The terrestrial input, remineralization of N within the system, and calculated MAD differ with these scenarios. All fluxes are labeled and values given in  $\text{g N m}^{-2} \text{yr}^{-1}$ . For values with 2 flux estimates, scenario 1 is listed first. (b) Implications of varying amounts of MAD for predictions of the  $\delta^{15}\text{N}$  value of organic material exported from the Hudson at river km 50. Predicted  $\delta^{15}\text{N}$  values are given for both scenario 1 (grey line and arrow) and scenario 2 (black line and arrow). The arrows represent our best estimates of MAD based on the bulk N budget (a). The dashed line at 12‰ represents the  $\delta^{15}\text{N}$  of seston exiting the Hudson at km 50 (Fig. 5).

and scenario 2 = soil humic scenario). Under scenario 1, terrestrial materials contribute  $9 \text{ g N m}^{-2} \text{yr}^{-1}$ . Under scenario 2, terrestrial materials contribute  $22 \text{ g N m}^{-2} \text{yr}^{-1}$ . Thus, the total organic N input to the system (without consideration of MAD) is 20 and 33 for scenarios 1 and 2 (Fig. 6a).

The losses of organic N by burial and export are 5 and  $20 \text{ g N m}^{-2} \text{yr}^{-1}$ , respectively. If all inputs are respired at the same proportion (ca 40% of inputs), the loss of organic N by within-system decomposition would equal 8 and  $13 \text{ g N m}^{-2} \text{yr}^{-1}$  for scenarios 1 and 2, respectively. Thus, under scenario 1 a MAD of about  $13 \text{ g N m}^{-2} \text{yr}^{-1}$  would be required to balance the organic N budget. Scenario 2, on the other hand, would require somewhat lower MAD ( $5.5 \text{ g N m}^{-2} \text{yr}^{-1}$ , Fig. 6a).

Like the bulk N budget, the  $^{15}\text{N}$  budget of the Hudson is also not in balance in the absence of MAD. Using scenario 1, the  $\delta^{15}\text{N}$  of the average material entering the Hudson is 0.5‰. For scenario 2 the average value is 4.5‰. Actual measurements on seston suggest that the  $\delta^{15}\text{N}$  of organic material leaving the river at km 50 is between 10 and 14‰ (Fig. 5a). If we assume this discrepancy is due to MAD from a DIN pool of 15‰ (above), we can calculate the impact of MAD on the  $^{15}\text{N}$  pool (Fig. 6b). This calculation suggests that under both scenarios a MAD of  $8 \text{ g N m}^{-2} \text{yr}^{-1}$  would result in a system average  $\delta^{15}\text{N}$  of near 7‰, while for both scenarios a MAD of  $13 \text{ g N m}^{-2} \text{yr}^{-1}$  would result in a system average  $\delta^{15}\text{N}$  of near 10‰. Thus, the bulk N demand of  $13 \text{ g N m}^{-2} \text{yr}^{-1}$  (scenario 1) most closely balances the  $^{15}\text{N}$  budget (Fig. 6b).

## DISCUSSION

### MAD in the Hudson

MAD both enriches detrital organic matter with N and adds new organic N to the ecosystem. Detrital material can, however, also be enriched during decomposition by conservation of initial N in decomposing material (Fig. 1). While the bulk N enrichments we observed cannot distinguish these possibilities well the isotope results from the *in situ* incubations suggest that MAD was the cause of the observed N enrichment (Fig. 2b).

In our *in situ* (litter bag) experiments, the  $\delta^{15}\text{N}$  of leaf material incubated for 3 mo in the Hudson showed a nearly 15‰ increase (Fig. 2c). As the  $\delta^{15}\text{N}$  values of autotrophs in the Hudson suggest that the DIN pool of the Hudson is heavy, MAD from this pool would be a feasible mechanism for driving the dramatic increases in  $\delta^{15}\text{N}$  during the *in situ* experiments. Other explanations for this dramatic enrichment are possible, however, and include both microbial assimilation of DON with high  $^{15}\text{N}$  values and preferential retention of some organic N components during microbial processing (Macko & Estep 1984).

While the *in situ* experiments are consistent with MAD, the microcosm experiments more clearly demonstrate that MAD can occur when terrestrially derived material is incubated in Hudson water. In these experiments, when organic N with initial  $\delta^{15}\text{N}$  of 10‰ was incubated with a  $^{15}\text{N}$  enriched DIN source (10000‰), PON and DON achieved  $\delta^{15}\text{N}$  values of



over 5000 and 1000‰, respectively. Thus, DIN clearly entered the organic N pool in the dark (when no autotrophy occurred). Further, the heavier PON pool as compared to DON pool suggests that the flow of DIN was first into particulate material and then into the DON pool.

If we assume that the isotope shift in both experiments was due solely to MAD, we can calculate the importance of MAD as

$$\%MAD(t) = 100 \frac{(^{15}N_{ON(i)} - ^{15}N_{ON(t)})}{(^{15}N_{ON(i)} - ^{15}N_{DIN})}$$

(Wada et al. 1993)

where  $\%MAD(t)$  is the amount of N in the material at time  $t$  that originated from MAD,  $N_{ON(t)}$  and  $^{15}N_{ON(i)}$  are the  $^{15}N$  values of the organic material at time  $t$  and initially, and  $^{15}N_{DIN}$  is the  $^{15}N$  of the DIN pool that is immobilized.  $^{15}N_{DIN}$  of 15 and 10 000‰ were used for the *in situ* and microcosm results, respectively.

For the microcosm experiment this approach leads to the conclusion that after 7 d the  $\%MAD$  in the PON material was nearly 30% and after 1 mo was nearly 50%. The DON in the same experiment increased to over 10% MAD. Similarly, in the *in situ* experiments the PON pool increased steadily to near 50% MAD after 1 mo (Fig. 7) and was nearly 70% by the end of the experiment. Conversely, the N that was initially in the leaf declined exponentially during the experiment

and showed similar but slightly faster rates of decrease than did the C in the leaf. Thus, without MAD the C:N ratio of leaf material evidently would have actually increased during the experiment from 70 to 90 rather than decreasing to near 25 (Fig. 7). It appears, therefore, that MAD in the Hudson occurs and may be critical in enriching N in decomposing material as preservation of initial N as compared to C does not occur.

In addition to enriching decomposing material in N, MAD, just as AAD, creates new organic N (Fig. 1). In the Hudson an ecosystem level budget suggests that MAD may, in fact, produce more organic N than does phytoplankton production. While these ecosystem budgets have large errors associated with them, the  $^{15}N$  budgets also suggest a relatively large MAD. Finally, the pattern of increasingly elevated  $^{15}N$  of seston going from up river (km 200) to down river (km 50) locations is consistent with MAD progressively enriching terrestrial inputs as they move downstream (Fig. 5).

### Comparison to MAD in other systems

In terms of MAD's importance to ecosystem organic N inputs, we know of no studies to which we can compare our budgetary findings from the Hudson (Fig. 6). In terms of enrichment of organic material in N, there are actually surprisingly few studies with which we can compare our results. This is because, in these terms, MAD has generally been inferred in aquatic and terrestrial systems from increases in bulk N during decomposition of plant material in litter bags (e.g. Meyer & Johnson 1983, White & Howes 1994, Coleman & Crossley 1996). The use of bulk N only, rather than bulk N and  $^{15}N$  content, can, however, cause severe underestimates of MAD (White & Howes 1994).

Compared to the few isotope based studies of MAD from forests and marsh lands (White & Howes 1994, Coleman & Crossley 1996), it appears that MAD in the Hudson is quite high. For example, it took nearly 1 yr for MAD to account for 50% of the N in decomposing *Spartina alterniflora* litter in a marsh system (White & Howes 1994); in the Hudson, by 1 mo 50% of the litter N originated from MAD. This higher MAD is associated with an overall high decomposition rate (in terms of total weight loss) in the Hudson (Fig. 1). The higher overall decomposition rates in the Hudson could be the result of high rates of MAD. Other researchers in diverse environments have shown more rapid decomposition rates of plant detritus in the presence of high levels of exogenous DIN (Carpenter & Adams 1979, Meyer & Johnson 1983). Other factors such as higher water availability (compared to terrestrial systems) or high grazing rates may also contribute to the rapid decomposition rates in the Hudson. In any case, our

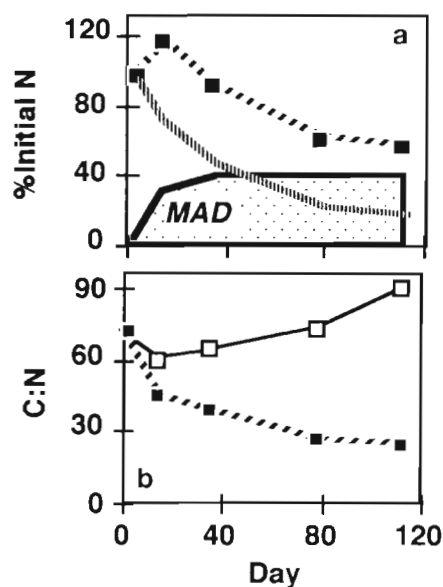


Fig. 7 Results derived from the *in situ* incubation in Fig. 1 (a) N content as divided into that originating from MAD and that from the oak leaves initially added to litter bags. Solid line is MAD derived N. Exponentially declining stippled line is original N and dashed line with boxes is total N. (b) Measured C:N of leaf material ( $\square$ ) and that modeled from the original N of the material ( $\blacksquare$ ). The modeled C:N represents what the C:N of the leaf material would be if MAD had not occurred

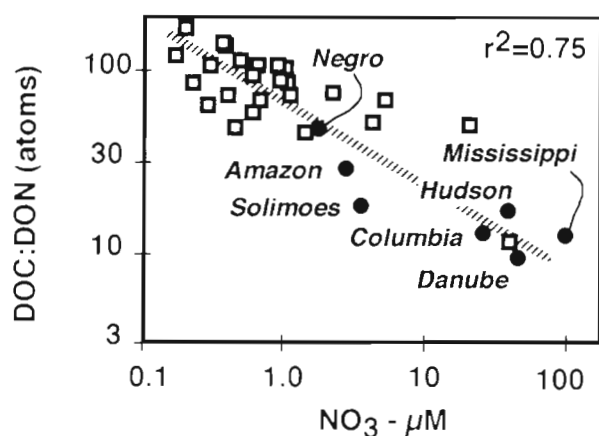


Fig. 8. C:N of DOM in rivers versus  $\text{NO}_3^-$  concentration in those same rivers. Data are Canadian rivers of the Atlantic Provinces ( $\square$ ; Clair et al. 1994) and large world rivers ( $\bullet$ ; Meybeck 1982, Esser & Kohlmaier 1991). The large rivers are labeled

results show that MAD in river systems can be high and is perhaps higher than in many other systems where this process is more routinely measured and thought to be of significance.

The high rate of MAD in the Hudson is possibly due to the high DIN levels, and MAD may be lower in other aquatic systems with lower DIN. Intriguing evidence that this may be an important control comes from a comparison of DOC:DON ratios in rivers with differing  $\text{NO}_3^-$  sources (Fig. 8). This comparison shows that this ratio varies dramatically across different river systems from near 80 to less than 20. Further, 75% of the variation in this ratio is explained by the  $\text{NO}_3^-$  concentration in the river water. As MAD can enrich the DOM pool in N, it is possible that differing amounts of MAD in these rivers are the mechanism behind this pattern.

### Implications of MAD

High MAD could have an impact on the DIN pool within aquatic systems. For the Hudson, however, this impact is likely to be small. The standing stock of DIN in the Hudson is approximately  $5 \text{ g N m}^{-2}$  and this DIN pool is renewed from outside inputs about 10 times per year (Caraco et al. 1997). Thus, a MAD of  $13 \text{ g N m}^{-2} \text{ yr}^{-1}$  is possible without a severe depletion of DIN within the system. Obviously, this same N demand would be far more significant to the depletion of DIN in a system with lower N supply and ultimately MAD in these systems could be limited by lack of DIN supply or would have to be fueled by fixation of  $\text{N}_2$  (White & Howes 1994, Currin et al. 1995). If MAD does indeed become limited by external DIN in low DIN systems it would have implications for patterns of food quality.

Aquatic scientists tend to think of nutrient content of organic material and food quality as being primarily controlled by its origin (e.g. terrestrial material vs algae). Microbial processing can dramatically alter food quality of organics and, if this process is dependent on dissolved nutrient content, the quality of the same oak leaf that entered a system with high DIN (and perhaps DIP; Howarth & Fisher 1976) could after 1 mo have far greater food quality than one that entered a low nutrient system. Interestingly, this nutrient dependent microbial processing could provide an alternative explanation for the previously observed relationship between inorganic nutrient concentrations and secondary production in aquatic systems (Hanson & Leggett 1982, Peters 1986, Currie 1991). As primary production is related to nutrient inputs or concentrations (Peters 1986), it is generally considered that the correlations between nutrient concentration and secondary production are driven by primary production increases at higher nutrient concentration (Cole et al. 1988, but see Currie 1991 for argument on bacteria). It is possible, however, that microbial assimilation of DIN and/or DIP could in part be driving the relationships between nutrients and microbes, nutrients and invertebrates, and nutrients and fish.

In addition to demonstrating that MAD can alter total N content of decomposing allochthonous material, our results also show that  $\delta^{15}\text{N}$  content can be altered. During decomposition within the Hudson,  $\delta^{15}\text{N}$  of leaf material increased by about 13‰. The instability of  $\delta^{15}\text{N}$  content has potentially large impacts on interpretation of isotope results in food web studies. MAD has generally not been considered in interpretation of isotope results (e.g. Hamilton et al. 1982, Deegan et al. 1990, Sullivan & Moncreiff 1990, Fry 1991, Wada et al. 1993). There are only a few studies that show that  $^{15}\text{N}$  signals can be altered during digestion in nature (Zieman et al. 1984, Thornton & McManus 1994, Kikuchi & Wada 1996). Further, the only food web study we know of that has explicitly investigated the impact of microbial processing on interpretation of isotope food web studies is that of Currin et al. (1995). Interestingly, this study on isotope changes in *Spartina alterniflora* showed the opposite impact of microbial processing on  $^{15}\text{N}$  content that we found. That is, during digestion standing dead *S. alterniflora* decreased in  $^{15}\text{N}$  content. This is partly due to the fact that the original  $^{15}\text{N}$  signal in live *S. alterniflora* was quite high as compared to that of the oak leaves we used ( $\delta^{15}\text{N}$  ca +4 and -4‰, respectively), but is also due to a different source of N for MAD. That is, the origin of the N in our study was probably dissolved  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the river with a heavy  $^{15}\text{N}$  signal, the origin in the study of Currin et al. (1995) was thought to be atmospheric  $\text{N}_2$  fixed with a  $\delta^{15}\text{N}$  signal of 0‰. This difference suggests that the

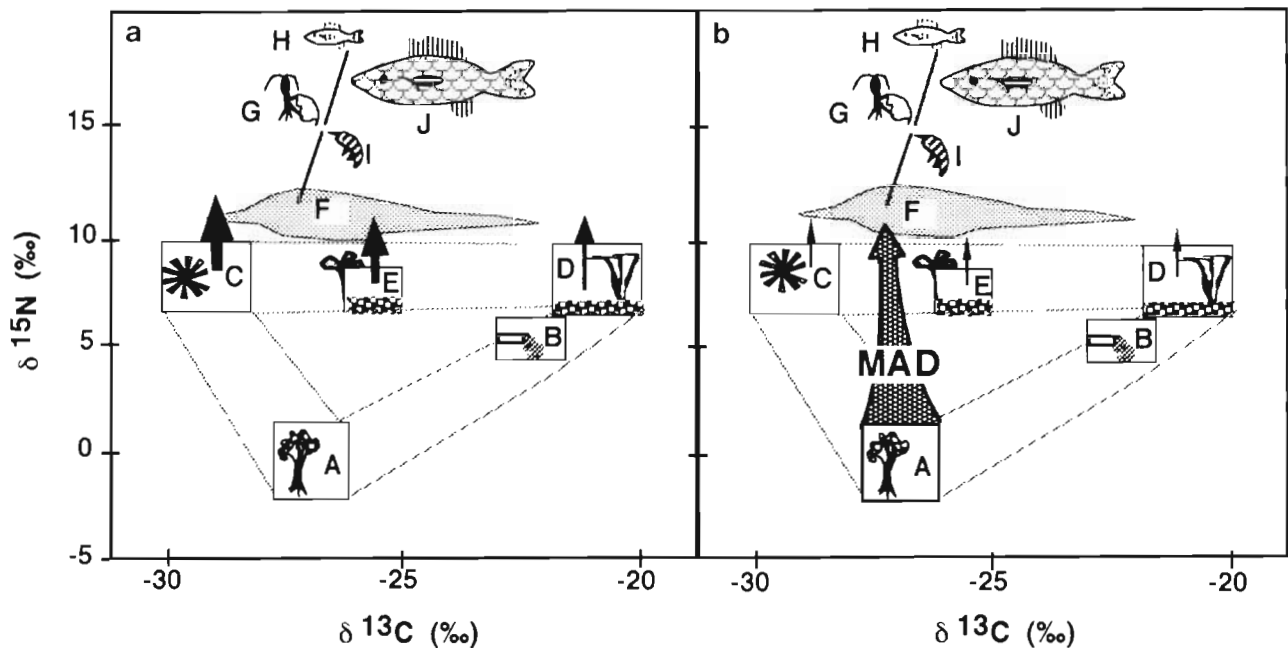


Fig. 9. Diagrams of material transfers based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content of producers and consumers in the Hudson River. We show here 2 scenarios for interpretation of stable isotope results. (a) The traditional analysis which assumes no microbial assimilation of DIN (MAD). This scenario would suggest that allochthonous material is unimportant to consumers and the organic N and C demand is met almost entirely from a mixture of phytoplankton and emergent and submerged macrophyte material. (b) Interpretation with MAD. This interpretation suggests that allochthonous organic C is important to consumers and most of the N comes from MAD within the system. Under this interpretation autochthonous production could have little contribution. Both scenarios can explain relatively well the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content of seston and most consumers in the Hudson. Labeled components of the system are: A, terrestrial material; B, sewage; C, phytoplankton; D, submerged macrophytes; E, emergent macrophytes; F, seston; G, zooplankton; H, larval fish; I, benthic invertebrates; and J, adult and juvenile fish. Sewage values (B) are averages for the NE USA as reported in Van Dover et al. (1992)

impact of MAD on the  $\delta^{15}\text{N}$  signal can be very complex, varying not only in magnitude but also direction in different aquatic systems.

Whether MAD results in increases or decreases in  $\delta^{15}\text{N}$  content of decomposing litter, the failure to recognize it can result in erroneous interpretations of isotope data. In their marsh study, Currin et al. (1995) found that the importance of *Spartina alterniflora* as a food source was underestimated if microbially induced changes in isotope content were not considered. In the case of the Hudson, ignoring MAD we would conclude, based on the high  $\delta^{15}\text{N}$  content of consumers (Fig. 4), that allochthonous terrestrial material was not important to the food web. Rather, a combination of phytoplankton and macrophytes would appear to be of dominant importance (C or N; Fig. 9a). When we consider the possibility of significant MAD, however, the data suggest that allochthonous material could be the most important C source for consumers, MAD the most important source of N, and phytoplankton and macrophytes could be relatively unimportant (Fig. 9b).

Microbial shifts in  $\delta^{15}\text{N}$  content could also affect interpretations in isotope studies that use N to look at

trophic position (even where there is only 1 initial organic source; Fry & Quinones 1994, Vander Zanden et al. 1996). The impact, however, could be complex. For example, if an invertebrate consumer ate microbially processed rather than live *Spartina alterniflora*, the work of Currin et al. (1995) suggests that it could be interpreted as feeding at a lower trophic level. In the Hudson, however, if an invertebrate ate microbially processed allochthonous material instead of fresh leaves it could be interpreted as being at up to 4 trophic levels higher in the food chain. Clearly, more work is needed in different systems to understand the actual impact of MAD.

In conclusion, several lines of evidence suggest that MAD is an important pathway of organic N production in the Hudson. MAD could be high in rivers and oligohaline sections of estuaries in general due to the high DIN in these systems. When MAD occurs it can both increase the N content of allochthonous material and alter the  $\delta^{15}\text{N}$  signal of this material. MAD may, therefore, not only give fish and invertebrates a 'reason' to consume more allochthonous organic matter but could also make detection of this consumption more difficult.

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#### LITERATURE CITED

- Allan JD (1995) Stream ecology: structure and function of running waters. Chapman & Hall, London
- Angradi TR (1994) Trophic linkages in the lower Colorado River: multiple stable isotope evidence. *J N Am Benthol Soc* 13:479–495
- Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of  $^{13}\text{C}$  in lignin and its implications for stable carbon isotope studies. *Nature* 329:708–710
- Boynton WR, Garber JH, Summers R, Kemp WM (1995) Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18:285–314
- Caraco NF, Cole JJ, Raymond PA, Strayer DL, Pace ML, Findlay SEG, Fischer DT (1997) Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. *Ecology* 78:588–602
- Carpenter SR, Adams MS (1979) Effects of nutrients and temperature on decomposition of *Meriophyllum spicatum* L. in a hard-water eutrophic lake. *Limnol Oceanogr* 24:520–528
- Cifuentes LA, Sharp JH, Fogel ML (1988) Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnol Oceanogr* 33:1102–1115
- Clair TA, Pollock TL, Ehrman JM (1994) Exports of carbon and nitrogen from river basins in Canada's Atlantic Provinces. *Global Biogeochem Cycles* 9:441–450
- Cole JJ, Caraco NF, Peierls BL (1992) Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary? *Limnol Oceanogr* 37:1608–1617
- Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar Ecol Prog Ser* 43:1–10
- Cole JJ, Peierls BL, Caraco NF, Pace ML (1993) Nitrogen loading of rivers as a human-driven process. In: McDonnell MJ, Pickett STA (eds) *Humans as components of ecosystems: the ecology of subtle human effects and populated areas*. Springer-Verlag, Berlin, p 141–157
- Coleman DC, Crossley DA Jr (1996) *Fundamentals of soil ecology*. Academic Press, San Diego
- Corner EDS, Davies AG (1971) Plankton as a factor in the nitrogen and phosphorus cycles in the sea. *Adv Mar Biol* 9:101–204
- Currie DJ (1991) Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. *Limnol Oceanogr* 37:1437–1455
- Currin CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Mar Ecol Prog Ser* 121:99–116
- Deegan LA, Peterson BJ, Portier R (1990) Stable isotopes and cellulase activity as evidence for detritus as a food source for juvenile gulf menhaden. *Estuaries* 13:14–19
- Esser G, Kohlmaier GH (1991) Modelling terrestrial sources of nitrogen, phosphorus, sulphur and organic carbon to rivers. In: Degens ET, Kempe S, Richey JE (eds) *Biogeochemistry of major world rivers*. Wiley & Sons, New York, p 297–322
- Findlay SF, Pace ML, Lints D, Cole JJ, Caraco NF (1991) Weak coupling of bacterial and algal production in a heterotrophic ecosystem: the Hudson River estuary. *Limnol Oceanogr* 36:268–278
- Findlay SF, Pace ML, Lints D, Howe K (1992) Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. *Mar Ecol Prog Ser* 89:147–153
- Findlay SF, Tenore KR (1982) Nitrogen source for a detritivore: detritus substrate versus associated microbes. *Science* 219:371–372
- Fonselius SH (1978) On nutrients and their role as production limiting factors in the Baltic. *Acta Hydrochim Hydrobiol* 6:329–339
- Fry B (1991) Stable isotope diagrams of freshwater food webs. *Ecology* 72:2293–2297
- Fry B, Quinones RB (1994) Biomass spectra and stable isotope indicators of trophic level in zooplankton of the northwest Atlantic. *Mar Ecol Prog Ser* 112:201–204
- Goericke R, Montoya JP, Fry B (1994) Physiology of isotopic fractionation in algae and cyanobacteria. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Science, London, p 187–221
- Hamilton SK, Lewis WM, Sippel SJ (1982) Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. *Oecologia* 89:324–330
- Hanson JM, Leggett WC (1982) Empirical prediction of fish biomass and yield. *Can J Fish Aquat Sci* 39:257–263
- Harley MT, Findlay S (1994) Photosynthetic-irradiance relationships for three species of submersed macrophytes in the tidal freshwater Hudson River. *Estuaries* 17:200–205
- Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. *Limnol Oceanogr* 38:709–724
- Hopkinson CS, Vallino JJ (1995) The relationships among man's activities in watershed and estuaries: a model of runoff effects on patterns of estuarine community metabolism. *Estuaries* 18:598–621
- Horrigan SG, Hagström Å, Koike I, Azam F (1988) Inorganic nitrogen utilization by assemblages of marine bacteria in seawater culture. *Mar Ecol Prog Ser* 50:147–150
- Howarth RW, Fisher SG (1976) Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. *Freshwat Biol* 6:221–228
- Howarth RW, Schneider R, Swaney D (1996) Metabolism and organic carbon fluxes in the tidal freshwater Hudson River. *Estuaries* 19:848–865
- Kikuchi E, Wada E (1996) Carbon and nitrogen stable isotope ratios of deposit-feeding polychaetes in the Nanakita River Estuary, Japan. *Hydrobiologia* 321:69–75
- Limburg KE (1998) Anomalous migrations of anadromous herrings revealed with natural chemical tracers. *Can J Fish Aquat Sci* 55:431–437
- Limburg KE, Moran MA, McDowell WH (1986) *The Hudson River ecosystem*. Springer Verlag, New York
- Mackenthum KM (1973) Eutrophication and biological associations. In: Griffith EJ, Beeton A, Spencer JM, Mitchell DT (eds) *Environment phosphorus handbook*. Wiley, New York, p 613–632
- Macko SA, Estep MLF (1984) Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Org Geochem* 6:787–790
- Mantoura RFC, Owens NJP, Burkil PH (1988) Nitrogen biogeochemistry and modelling of Carmarthen Bay. In: Blackburn TH, Sorensen J (eds) *Nitrogen cycling in*

- coastal marine environments. Wiley & Sons, Chichester, p 415–441
- McQuire AD, Melillo JM, Joyce LA (1995) The role of nitrogen in the response of forest net primary production to elevated atmospheric carbon dioxide. *Annu Rev Ecol Syst* 26: 473–503
- Meybeck M (1982) Carbon, nitrogen, and phosphorus transport by world rivers. *Am J Sci* 292:401–450
- Meyer JL, Johnson C (1983) The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwat Biol* 13:177–183
- Michener RH, Schell DM (1994) Stable isotopic ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Science, London, p 138–157
- Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Science, London, p 138–157
- Oviatt C, Doering C, Nowicki B, Reed L, Cole J, Frithsen J (1995) An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. *Mar Ecol Prog Ser* 116:171–179
- Pace ML, Findlay SEG, Lints D (1992) Zooplankton in advective environments: the Hudson River community and a comparative analysis. *Can J Fish Aquat Sci* 49:1060–1069
- Peters RH (1986) The role of prediction in limnology. *Limnol Oceanogr* 31:1143–1159
- Peterson BJ, Hobbie JE, Corliss TL (1986) Carbon flow in a tundra stream ecosystem. *Can J Fish Aquat Sci* 43: 1259–1270
- Peterson BJ, Howarth RW, Garritt RH (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227:1361–1363
- Raymond PA, Caraco NF, Cole JJ (1997) CO<sub>2</sub> concentration and atmospheric flux in the Hudson River. *Estuaries* 20: 381–390
- Richey JE and 8 others (1978) Carbon flow in four lake ecosystems: a structural approach. *Science* 202:1183–1186
- Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385: 61–64
- Strayer DL, Powell J, Ambrose P, Pace ML, Fischer DT (1996) Arrival, spread, and early dynamics of a zebra mussel (*Dreissena polymorpha*) population in the Hudson River estuary. *Can J Fish Aquat Sci* 53:1143–1149
- Suberkropp K, Chauvet E (1995) Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology* 76:1443–1445
- Sullivan MJ, Moncreiff CA (1990) Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analysis. *Mar Ecol Prog Ser* 62:149–159
- Swaney DP, Sherman D, Howarth RW (1996) Modeling water, sediment and organic carbon discharges in the Hudson-Mohawk Basin: coupling to terrestrial sources. *Estuaries* 19:833–847
- Tenore KR (1988) Nitrogen in benthic food chains. In: Blackburn TH, Sorensen J (eds) *Nitrogen cycling in coastal marine environments*. Wiley & Sons, Chichester, p 190–206
- Thornton SF, McManus J (1994) Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. *Estuar Coast Shelf Sci* 38:219–233
- Van Dover CL, Grassle JF, Fry B, Garritt RJ, Starczak VR (1992) Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature* 360:153–155
- Vander Zanden MJ, Cabana G, Rasmussen JB (1996) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) and literature dietary data. *Can J Fish Aquat Sci* 54:1142–1158
- Vitousek PM (1984) Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* 65:285–298
- Wada E, Kabaya Y, Kurihara Y (1993) Stable isotopic structure of aquatic ecosystems. *J Biosci* 18:483–499
- Welcomme RL (1995) River fisheries. *FAO Fish Techn Rep* 262
- Wheeler PA, Kirchman DL (1986) Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol Oceanogr* 31:998–1009
- White DS, Howes BL (1994) Nitrogen incorporation into decomposing litter of *Spartina alterniflora*. *Limnol Oceanogr* 39:133–139
- Zieman JC, Macko SA, Mills AL (1984) Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bull Mar Sci* 35:380–392

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