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Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton

Abstract—Atmospheric deposition is recognized as a potentially large source of inorganic nutrients to many ecosystems. In marine systems, where nitrogen (N) is the nutrient typically limiting phytoplankton growth, rainwater is often a significant source of N. Although a considerable portion of atmospheric N deposition is in the form of organic N, only the inorganic N in rainwater has been considered by most previous studies. Laboratory experiments presented here indicate that dissolved organic nitrogen (DON) from rainwater can stimulate productivity of coastal marine bacteria and phytoplankton. A large percent of rainwater DON was potentially biologically available; 45–75% was rapidly utilized by the microorganisms. The magnitude of the response of bacterial and phytoplankton biomass to equivalent amounts of DON-N or ammonium-N was similar. However, the community composition of phytoplankton at the end of the experiment differed in treatments receiving DON and inorganic N. Diatoms and dinoflagellates accounted for >90% of the phytoplankton biomass in treatments receiving rainwater DON. In contrast, small (<2 μm) monads accounted for >85% of the biomass in the treatments receiving ammonium. The results indicate that DON in rainwater can be an important source of N to ecosystems.

Atmospheric deposition is currently a major source of N to many aquatic and terrestrial ecosystems (Fisher et al. 1988; Howarth et al. 1996; Jaworski et al. 1997). Atmospheric N deposition is predicted to increase in the future due to human activities; deposition of NOy from fossil-fuel combustion alone is predicted to increase by up to a factor of four in some geographical regions by the year 2020 (Galloway et al. 1994). Because N is the primary nutrient-limiting plant, algal and microbial production in many terrestrial and marine environments, as well as some freshwater environments, increases in N inputs can markedly alter those ecosystems (Ryther and Dunstan 1971; Schlesinger 1991; Vitousek and Howarth 1991). To date, measurements and models of atmospherically deposited N, as well as studies of its effects on ecosystems, have focused on inorganic N (e.g., nitrate and ammonium) (Paerl 1985; Galloway et al. 1994; Prospero et al. 1996; Wedin and Tilman 1996). However, data from a wide range of locations demonstrate that organic N is also an important component of N in rainwater and ranges from approximately 20 to 70% of the total N deposited annually (Table 1).

The sources and chemical composition of organic N in rainwater are poorly characterized (Cornell et al. 1995; Gorzelska et al. 1997; Cornell et al. 1998). In addition, we are only just beginning to obtain information on the biological availability of organic N in rainwater and its effects on ecosystems. While rainwater has been shown to stimulate phytoplankton production in coastal and open-ocean regions (Paerl 1985; Paerl et al. 1990; Owens et al. 1992; Zhang 1994), these studies do not document utilization of rainwater organic N or bacterial and/or phytoplankton stimulation specifically due to the organic N inputs. In a recent study, DON from rainwater collected in coastal North Carolina was shown to stimulate phytoplankton production and, in some cases, biomass over short (24–48 h) time scales (Peierls and Paerl 1997). Axenic cultures of freshwater algae also were stimulated by DON in rainwater from New Zealand and Japan (Timperley et al. 1985). The purposes of the current study were to examine: (1) how much of the DON in rainwater collected from a coastal urban area is potentially available for use by estuarine plankton, and (2) what the potential effect of the rainwater DON is on bacterial and phytoplankton production and biomass as well as on plankton community composition.

Rainwater was collected in Philadelphia, Pennsylvania, using acid-cleaned plastic containers. These were placed outside (elevated 1 m above roof of four-story building) within 1 h before a rain event and collected within 1 h of the end of the event. The water was immediately filtered through precombusted (450°C, 4 h) Whatman GF/F glass-fiber filters and stored frozen in fluorinated, high-density polyethylene 5- or 10-liter carboys until use. Subsamples were frozen separately from each rain event for subsequent analysis of ammonium, nitrate plus nitrite, and total dissolved N (*see below for methods*). Procedural blanks of distilled-deionized water were analyzed for ammonium, nitrate, and dissolved organic C and N to check for possible contamination from the collection and storage procedures. No measurable contamination was detected.

The biological availability of DON in rainwater and its effects on coastal plankton communities were examined with two different types of experiments. In the first experiment, concentrates of estuarine bacteria were added to sterile-filtered rainwater, and the growth of bacteria and decreases in DON concentration were followed over time. Rainwater collected during two storm events in June 1992 were combined and used for this experiment. The experimental approach follows a methodology used to examine the biological availability of DON in river water (Seitzinger and Sanders 1997). The thawed rainwater was filter sterilized (0.2-μm spiral-wound glass-fiber filters) and adjusted to an estuarine salinity of 15 ppt using a combination of precombusted salts (Kester et al. 1967); the rainwater was maintained at 4°C during filtering. (We have no direct information on the effects of freezing and thawing on the biological availability of the rainwater DON. However, previous experiments demonstrated a high degree of biological availability of DON in river water that had not been previously frozen; [Seitzinger and Sanders 1997].) Controls consisted of deionized water adjusted to a salinity of 15 ppt. Concentrates of estuarine bacteria were prepared using water (15 ppt, from the small estuary Barnegat Bay, New Jersey), which was sequentially

Table 1. Percent of total N as organic N in precipitation from various locations. Annual averages or ranges are shown when available.

Source/Location	% org N	Reference	Comment
Walker Branch, TN	34	Kelly and Meagher 1986	Annual average
Coastal plain, FL	40–63	Riekert 1983	Range of annual averages for 4 yr
Cascade Mtns., OR	46–72	Fredriksen 1976	Range of annual average for 4 yr, site HJA-10
Coastal plain, SC	49	Richter et al. 1983	2-yr average
Philadelphia, PA*	19–52	This study	Range measurements Mar–Oct 1993; $n = 7$
Chesapeake Bay	57	Smullen et al. 1982	Annual data from six locations above head of tide
Rhode River, MD	18–44	Jordan et al. 1995	Range of annual averages for 14 yr
New Brunswick, NJ*†	2–44	Seitzinger and Sanders unpubl. data	Range of measurements made over 2.5 yr
Narragansett, RI	19	Nixon et al. 1995	Annual average
U.K.	21	Cornell et al. 1995	Single measurement
Czech Rep.	27	″	″
N. Carolina	21	″	″
Amazonia	22	″	″
Recife, Brazil	25	″	″
Bermuda	59	″	″
Tahiti	84	″	″
NE Atlantic	62	″	″
NE Atlantic	67	″	″
Cape Cod, MA	43	Valiela et al. 1997	2-yr average
Lewes, DE	23	Scudlark et al. 1998	Approximately 1-yr average

* Selected samples were analyzed for urea N, which was found to be a minor component (<5%) of the DON.
† Samples were collected and analyzed as described for rainwater samples in the current study.

filtered through a 35-μm mesh plankton net and a 1-μm Nuclepore polycarbonate filter. The bacteria were then concentrated from the filtrate using a 1,000-kd polysulphone filter (tangential flow ultrafiltration; Filtron Technologies). Protists were eliminated from the bacterial concentrate using a sonication step that eliminated remaining protists without significantly affecting bacterial growth (Seitzinger and Sanders 1997). No protists were observed for the duration of the experiment, which suggests that the treatment was effective for at least 10 d. Samples for protists were preserved with buffered 10% glutaraldehyde, stained with primulin, and counted via epifluorescence microscopy (Sherr et al. 1993; Sherr and Sherr 1993). Aliquots (~18 ml) of the bacterial concentrate were added to 10 liters of rainwater and 10 liters of control water to give an initial abundance of 2–4 $\times$ 10^5 cells ml^{-1}. Initial samples for nutrients and bacteria were taken from the rain- and control waters, then 4 liters of each was poured into duplicate 4-liter Erlenmeyer flasks (i.e., two control flasks and two rainwater flasks; 4 liters $flask^{-1}$). 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.

Time-series samples of water were taken over a 10-d period for NH_4^+, $NO_2^- + NO_3^-$ (Parsons et al. 1984; Alpkem 1991), soluble reactive phosphate (phosphate; Parsons et al. 1984), and total dissolved N (TDN; Walsh 1989). DON was determined by the difference between TDN and dissolved inorganic N (DIN: NH_4^+, $NO_2^- + NO_3^-$). TDN samples were analyzed by high-temperature combustion followed by chemiluminescent detection of nitric oxide (Walsh 1989) using a model 7000 total N analyzer (Antek) equipped with a quartz combustion tube (1,000 ± 10°C) and a ceramic insert.

Samples were analyzed for bacterial abundance (Hobbie et al. 1977) and biovolume (Lee and Furhman 1987). Absence of heterotrophic flagellates was confirmed using epifluorescent microscopy. 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. 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). Bacterial growth on flask walls was minimal, as determined from bacterial counts on glass cover slips suspended in the incubation flasks (Seitzinger and Sanders 1997).

The second type of experiment consisted of adding concentrates of rainwater DON to estuarine water and following the response of the plankton community and changes in DON and DIN concentrations. Concentrates of DON from rainwater (collected from five rain events between March and October 1993 and stored frozen) were prepared by low-temperature (60°C) vacuum evaporation (Timperley et al. 1985), followed by inorganic N removal using ion retardation columns (Bronk and Glibert 1993); the efficiency of recovery of DON was 54%. The final concentrate was divided into aliquots and frozen.

Freshly collected Barnegat Bay water was filtered through a 160-μm sieve to remove macrozooplankton. Six 1-liter volumes of bay water were incubated in Pyrex flasks at 20°C on a 12 : 12 light : dark (LD) cycle (light level 270 $\mu E\ m^{-2}\ s^{-1}$; photosynthetically active radiation [PAR]). The water was stirred slowly by Teflon-coated stir bars. Two sets of duplicate flasks received the same amount of either rainwater DON-N or NH_4^+-N (as ammonium chloride) approximately daily (4.7 μmol N $flask^{-1}\ d^{-1}$) for 13 d. Controls consisted of bay water without N additions. All microcosms received 0.21 μmol phosphate $flask^{-1}\ d^{-1}$ (as KH_2PO_4), so that the plankton would not become phosphorus limited. Phosphorus

concentrations in rainwater are very low relative to N and thus could potentially result in short-term P limitation in coastal ecosystems during a large rain event. However, there are numerous sources of P to coastal systems (e.g., point sources, runoff, and rivers), many of which also would increase during a rain event. Therefore, while it is possible that P additions increased utilization rates of DON in this bioassay experiment, P was added to simulate what we believe to be a more realistic representation of overall conditions in estuarine waters.

Time-series samples of the microcosm water were collected on days 1, 6, 9, 13, and 16 and analyzed for inorganic and dissolved organic N, phosphate, chlorophyll *a* (Chl *a*) concentration (Strickland and Parsons 1972), rates of primary production (^{14}C method, modified from Strickland and Parsons 1972), bacterial production (^{3}H-thymidine incorporation; Fuhrman and Azam 1982), and bacterial abundances. For primary production measurements, duplicate 15-ml samples plus dark controls were incubated for 2 h at the same light conditions and temperature as the microcosms. For bacterial production, four 10-ml samples were incubated for 30 min, with duplicate samples extracted with either hot or cold tricarboxylic acid. Radioactivity of the samples was determined by liquid scintillation counting (model LS6000IC, Beckman). Final samples were fixed with Lugol's iodine (1% v/v) and analyzed for plankton community composition from duplicate 100-ml settled samples using a Zeiss Axiovert inverted microscope. Phytoplankton biovolume was estimated based on microscopic counts, and cell sizes were measured using an ocular micrometer. DON utilization was calculated as the difference between the DON measured and the summed initial bay water plus added rainwater DON, after correcting for DON removed during sampling.

The current study suggests that a substantial portion of DON in rainwater can be biologically available to estuarine plankton communities. In the first experiment, we investigated the ability of estuarine bacteria to use the DON in rainwater as their sole source of nutrition. There was a rapid decrease in rainwater DON concentrations during the first 3 d of the experiment (Fig. 1). Rates of DON decrease averaged 2.6 μmol liter^{-1} d^{-1} over the first 3 d, resulting in a 46% decrease in initial rainwater DON concentrations. After day 3, DON remained relatively constant through the end of the experiment. DON concentrations in the control water remained below 3 μM throughout the experiment, demonstrating that any DON contained in the bacterial concentrate added at the beginning of the experiment was minimal and could not account for the DON decrease observed in the rainwater. DIN concentrations in the rainwater decreased by approximately 3 μM during the first 3 d and then remained relatively constant through day 8 (Fig. 1).

Bacterial abundances increased rapidly during the first 3 d of the experiment in the rainwater treatment by 15-fold (Fig. 1). Bacterial abundances in controls remained low and relatively constant throughout the experiment ($4.0 \times 10^8 \pm 0.2 \times 10^8$ cells liter^{-1}). The bacterial growth rate on rainwater dissolved organic matter averaged 6.4×10^5 cells ml^{-1} d^{-1} during the first 3 d, with a cell-specific growth rate (μ) of 1.22 d^{-1} calculated for the 2-d period of most rapid growth. This rate is within the range of bacterial growth

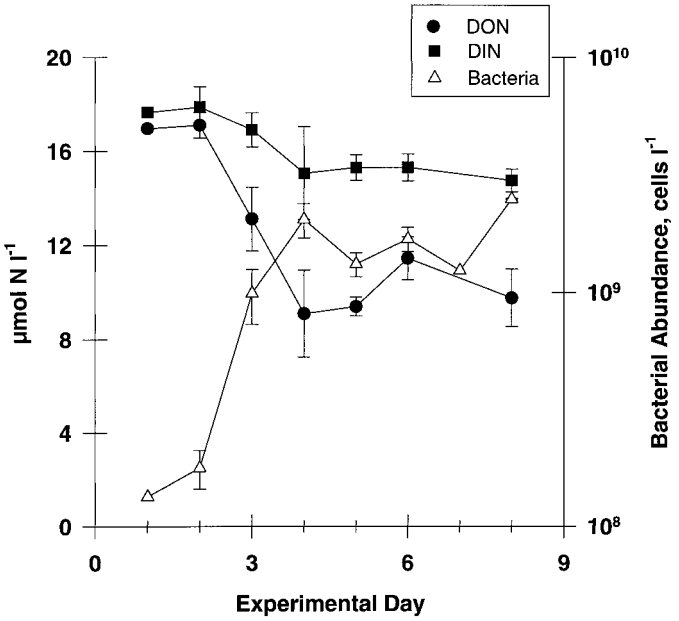

Fig. 1. Decrease in the concentration of rainwater DON and DIN (= ammonia + nitrate + nitrite) and resultant increase in bacterial abundance in filter-sterilized rainwater (salinity brought to 15 ppt), which had been inoculated with estuarine bacteria. Means ± SD of data from duplicate flasks of rainwater are shown.

observed in previous estuarine studies (*see* Seitzinger and Sanders 1997). This experiment was not meant to simulate the precise bacterial dynamics in estuaries resulting from rainwater DON inputs but rather, to examine the potential bioavailability of rainwater DON to estuarine bacteria. The similarity between rates of bacterial growth in this experiment and that in estuaries where there are numerous sources of dissolved organic matter simply indicates that our experimental results were ecologically reasonable.

The rapid decline in DON concentration during the first 3 d of incubation, which was mirrored by increases in bacterial abundance (Fig. 1), suggests that the DON was incorporated into bacterial biomass. This is further supported by N mass-balance calculations. Bacterial N calculated from biovolume and abundance was 9.2 μM N at the end of the experiment, which was similar to the decrease in rainwater DON (8 μM N) plus DIN (3 μM) (Fig. 1). We cannot rule out the possibility that some DON could have been lost through abiotic processes (e.g., sorption, flocculation, and volatilization); however, we have no indication that such processes were occurring. Rather, the combined DON and bacterial data suggest that approximately half of the DON in the rainwater was biologically available to the estuarine bacteria.

In the second type of experiment, in which concentrated rainwater DON was added to estuarine water, there also was a rapid disappearance of rainwater DON in concert with increases in growth and production of bacteria and phytoplankton (Figs. 2, 3). The increasing difference between the cumulative amount of DON added and the amount of DON remaining in the rainwater addition treatments during the first 9 d of additions indicates rapid utilization of rainwater

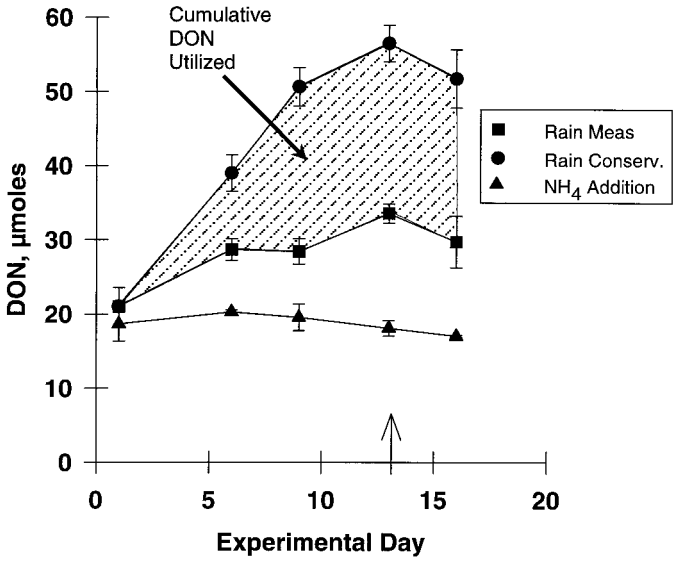

Fig. 2. Amount of DON measured and the cumulative amount of rainwater DON added to coastal plankton bioassays. Shaded area indicates the cumulative amount of DON utilized, which was calculated as the difference between the amount of DON added and the amount remaining over time. Also shown is the amount of DON in treatments receiving NH_4^+ additions. Data are means ± SD of duplicate flasks. Arrow indicates last day of N addition. The noticeable decrease in the measured and predicted amount of DON between days 13 and 16 is due to DON removed during sampling.

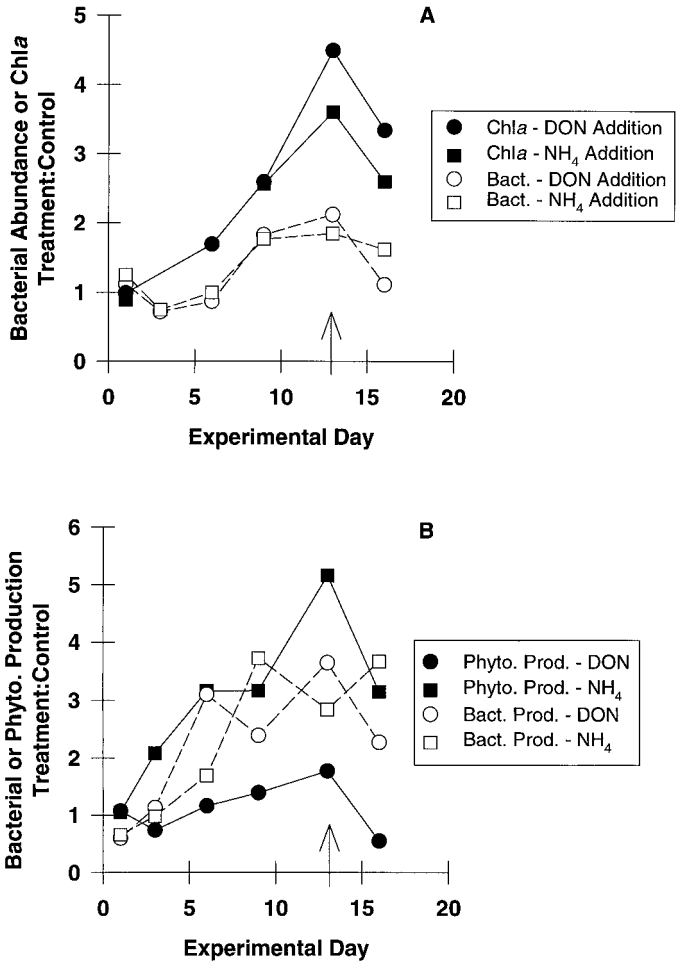

Fig. 3. Bacteria and phytoplankton response to either rainwater DON or ammonia additions. Data are reported relative to controls (bay water with no N addition). Arrow indicates last day of N addition. (A) Changes in bacterial abundance and phytoplankton Chl *a* concentration. (B) Changes in bacterial production and phytoplankton production.

DON (Fig. 2). By day 6, 58% of the DON added had been utilized, while by day 9, 75% had been utilized. However, it appears that there was no further net uptake of DON, because by the next measurement (day 13), the difference between the amount of DON added and DON remaining was essentially the same as on day 9 (23 μmol). Three days after DON additions stopped, the difference between the amount of DON added and remaining was still approximately 23 μmol, which suggests that some other factor had become limiting and/or that the remaining DON was relatively recalcitrant. There was no significant decrease in DON concentration over the course of the experiment in either control microcosms (15.3 ± 2.5 μM) (data not shown) or ammonium addition microcosms (16.5 ± 1.3 μM) (Fig. 2), suggesting that DON initially in the bay water was recalcitrant and/or was replaced by internal DON production.

The calculated utilization of rainwater DON was consistent with the measured changes in the bacteria and phytoplankton production and biomass, as well as with the changes in the phytoplankton community composition. Both bacteria and phytoplankton biomass (Fig. 3A) and production (Fig. 3B) increased relative to controls (with no N addition) during the period of rainwater DON additions. By the last day of DON additions (day 13), bacterial production was approximately threefold higher and abundance approximately twice as large, relative to controls. Phytoplankton production and particularly phytoplankton biomass (measured as Chl *a*) also were greater in the DON additions relative to controls. There was essentially no increase in bacterial abundance and production after day 9, which suggests that factors in addition to dissolved organic matter became limiting to the bacteria (e.g., predation and toxic component of rainwater addition). The pattern in bacterial abundance and production between days 9 and 13 is consistent with the DON data, indicating no further net utilization after day 9 (Fig. 2). However, the continued increase in phytoplankton production and biomass between days 9 and 13 indicates that whatever factor was limiting bacteria was not limiting the phytoplankton during that time. After cessation of DON additions, there was a decrease in phytoplankton biomass and production and in bacterial abundance, which further indicates the link between the DON additions and the biological responses measured.

Inorganic N remained below 1 μM in the DON (and inorganic N and control) treatment (data not shown), which indicates that any DON mineralized by bacteria and/or bacterivorous protozoa was immediately utilized. Some DON also may have been utilized directly by phytoplankton (*see below*).

As expected, ammonium addition resulted in increased bacterial and phytoplankton production and abundance/biomass (Fig. 3A,B). Stimulation of bacterial productivity and biomass and phytoplankton biomass (chlorophyll) per unit of DON-N or ammonium N addition were similar. This is consistent with the high percent of DON-N disappearance (~75%), which is attributed to plankton utilization. There was a decrease in phytoplankton biomass and production after ammonium additions stopped, similar to the decrease noted after DON additions stopped. This pattern of decrease after cessation of N (ammonium or DON) additions is consistent with phytoplankton and bacteria biomass and production in controls (with no N addition), which decreased throughout the experiment. This was expected, based on previous (inorganic) nutrient-addition experiments using 60-liter microcosms of Barnegat Bay water, which showed N limitation of phytoplankton (Seitzinger et al. 1993). In those summer experiments, N, or N plus P additions, stimulated phytoplankton production and biomass; however, controls (no additions) and P only additions resulted in phytoplankton decreases.

An obvious difference between the ammonium and DON treatments is the trend in primary production during the period of additions, which showed only a weak response to DON-N additions compared to the response to the same amount of ammonium-N additions (Fig. 3). This difference is reflected in the coefficient of assimilation or assimilation numbers (production rate : chlorophyll content). In the ammonium treatment, the assimilation number remained around 3 throughout the experiment. However, in the DON treatment, the assimilation ratio decreased to ca. 1. Both of these are within the range of assimilation numbers reported in the literature. Assimilation numbers for microplankton range from 0.2 to 20 mg C (mg Chl *a*)$^{-1}$ h^{-1}, and for picoplankton, they range from 0.03 to 15 mg C (mg Chl *a*)$^{-1}$ h^{-1} (Stockner and Antia 1986; Kelly 1989; Valiela 1995). A number of factors can affect the assimilation number (Falkowski and Raven 1997). The differences that we observed in assimilation numbers between treatments may reflect changes in one or more parameters (e.g., physiological condition, species composition, and food web dynamics). Further experiments would be required to determine the reasons for the differences we observed in assimilation ratios.

DON vs. ammonium additions resulted in large differences in the plankton community composition. At the end of the experiment, cell abundances of dinoflagellates, small monads (1.5–2.5 μm), and other flagellates were similar in control and DON treatments; diatoms were approximately four times more abundant in the DON treatment relative to the controls (Fig. 4A). In the ammonium treatment, however, no diatoms were observed, dinoflagellates were five times less abundant, and monads were approximately 20 times more abundant than in controls or DON treatments.

Within these phytoplankton groups, there were also differences. In the rainwater DON treatment, *Nitzschia closterium* was the most common diatom, but several species of *Navicula* and *Synedra* also were observed. *N. closterium* was the only diatom species observed in controls, and it tended to be smaller than those in the rainwater treatment. A small (<10 μm) *Gymnodinium* sp. was the numerically dominant

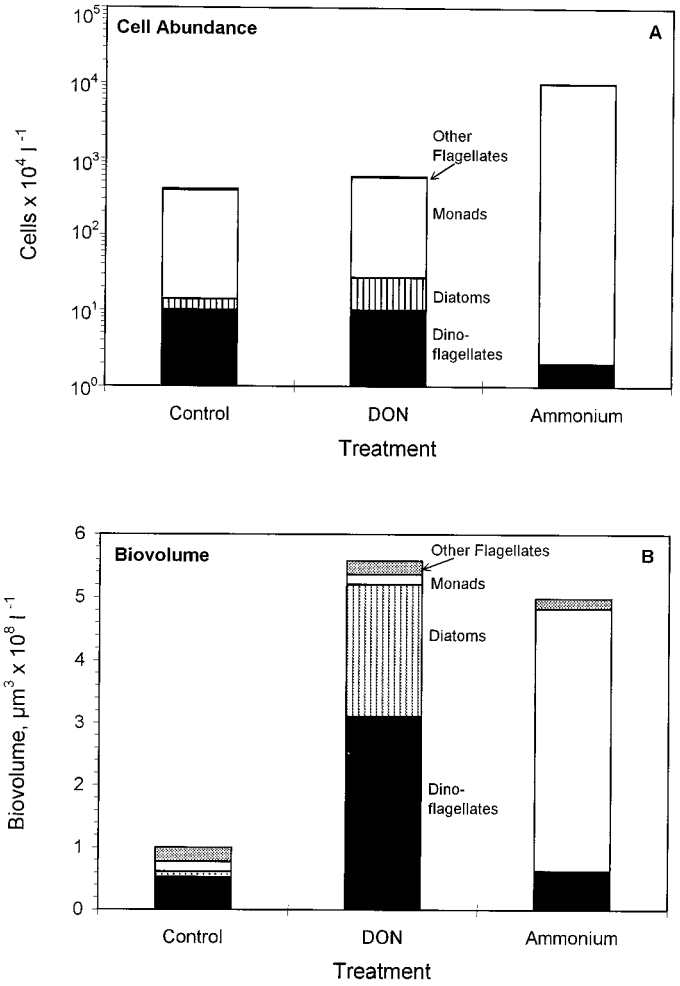

Fig. 4. Community composition at end of whole plankton experiment in control, rainwater DON, and ammonium treatments. Controls did not receive N additions (*see text*). (A) Cell abundance. (B) Biovolume.

dinoflagellate in the control and was codominant with the larger *Peridinium* (cf.) *brevipes* (ca. 25 μm) in the DON treatment. *Prorocentrum* sp. and *Dinophysis* sp. were present but rare in all samples.

The combined differences in cell numbers and species composition, as well as differences in size of *N. closterium* in controls compared to DON treatments, resulted in biovolume patterns among the treatments that were not reflected in cell counts alone. As noted above, the rainwater DON treatment had relatively large diatoms and dinoflagellates that were more abundant than in the control or ammonium treatments; small monads (1.5–2.5 μm) were more abundant in the ammonium treatment. A larger proportion of the phytoplankton biomass, therefore, was in relatively large cells (diatoms and dinoflagellates) in the rainwater DON treatment compared to the inorganic N treatment or the control (Fig. 4B). Biovolume estimates suggest that diatoms and dinoflagellates accounted for >90% of the phytoplankton biovolume in the rainwater DON treatment. In contrast, in the ammonium treatment, monads accounted for >85% of the biovolume. In the control, dinoflagellates and other fla-

gellates dominated the biovolume. Total phytoplankton biovolume in both rainwater DON and ammonium treatments was similar and about five times greater than the control. This is consistent with (1) the total phytoplankton biomass as measured by chlorophyll concentrations, which showed similar chlorophyll concentrations in DON and ammonium treatments (Fig. 3A), and (2) the high percent of DON utilization, which would have resulted in similar amounts of N available in both the ammonium and DON treatments.

The difference in the relative species composition and biomass of the phytoplankton between the DON and ammonium treatments indicates that DON additions were either directly or indirectly beneficial to the dinoflagellates and diatoms. This is in line with the known ability of some dinoflagellates and diatoms to utilize dissolved organic matter through uptake of low-molecular-weight compounds (Antia et al. 1991); in addition, some phytoplankton can oxidize amino acids and amines by cell-surface enzymes, thereby providing a source of ammonium for uptake (Pantoja and Lee 1994). Utilization of DON without prior mineralization by the microbial food web in the DON treatment may have given an advantage to the dinoflagellates and diatoms over the monads in competition for N. In contrast, it may be indirect effects associated with microbial food web dynamics that explain the higher abundances of dinoflagellates and diatoms in the DON treatment. Some insight into the complexity of the response of phytoplankton communities to DON additions is indicated by the range of responses reported in the literature at both the community level and in single species cultures (Antia et al. 1991; Paerl 1991; Carlsson and Granéli 1993; Carlsson et al. 1995).

It is not clear whether atmospheric DON could initiate or sustain an algal bloom. However, the ability to use DON preferentially has been implicated in harmful algal blooms (HAB), notably by the brown tide organism *Aureococcus anophagefferens* (Berg et al. 1997; LaRoche et al. 1997). Some species of dinoflagellates also form HABs. While none of the dinoflagellate species we observed in our experiments are known to be responsible for HABs, the rainwater DON did stimulate dinoflagellate growth.

In addition to differences in phytoplankton species composition, differences in grazer abundances were noted between the DON and ammonium treatments. Ciliated protozoa (almost entirely hypotrichs) were almost nine times more abundant in the ammonium treatment (26×10^4 cells liter^{-1}) compared to controls or DON treatment ($3.0 \pm 0.4 \times 10^4$ cells liter^{-1} and $3.0 \pm 0.1 \times 10^4$ cells liter^{-1}, respectively). The presence of monads in the ciliates' food vacuoles in the ammonium treatment demonstrated that they were grazing on these small phytoplankton. Most of the diatoms and dinoflagellates were as large or larger than the ciliates and were unlikely to be ingested. Protozoa were the only potential predators observed in the experiments. This would result in grazing pressure on the monads in controls, DON, and ammonium treatments but not on the larger diatoms and dinoflagellates in the controls or DON treatment.

Previous analyses have suggested that the atmosphere is an important source of N to a range of marine ecosystems (Fisher et al. 1988; Hinga et al. 1991; *see* summary in Paerl 1997). However, most studies have considered only atmospheric DIN inputs. Those studies that included both DIN and DON (Cornell et al. 1995; Nixon et al. 1995; Valiela et al. 1997; Scudlark et al. 1998) did not assess how much of the DON was biologically available. The current study suggests that development of biologically available N budgets for ecosystems requires measurements of both inorganic N deposition and that component of organic N deposition that is bioavailable. This is consistent with the growing number of studies that demonstrate the dynamic role of dissolved organic matter in aquatic ecosystems. A substantial portion of the dissolved organic matter entering aquatic systems from a range of sources has been found to be bioavailable (Timperley et al. 1985; Meyer et al. 1987; Carlsson and Granéli 1993; Amon and Benner 1994; Carlsson et al. 1995; Seitzinger and Sanders 1997). In addition, a significant portion of dissolved organic matter within aquatic systems can be readily used by aquatic microbes (Kirchman et al. 1991; Bronk et al. 1994, 1998; Gardner et al. 1996). Development of a biologically available N budget for any ecosystem will require considerably more data on the bioavailability of organic N in atmospheric deposition (as well as in other natural and pollutant N sources) than is currently available.

In the meantime, we can begin to explore the contribution of bioavailable atmospheric deposition to N loading for a variety of ecosystems by combining (1) rates of atmospheric N deposition of DIN and DON in different geographic locations (Table 1), (2) the results of the current study, which suggest that 45–75% of the DON in atmospheric deposition is biologically available, and (3) N inputs to specific ecosystems from sources other than the atmosphere. We have done this for six estuarine and coastal systems, four continental shelf regions in the North Atlantic, and the open oceans as a whole (Table 2). For the six coastal ecosystems, we compared the relative magnitude of atmospherically deposited bioavailable DON (DON_b) to total N inputs from sewage, agriculture, and the atmosphere (which includes atmospheric deposition on water surface plus export from watershed). In general, we used the results from, or applied the approach of, Hinga et al. (1991) to estimate the magnitude of N export from different sources for each watershed. For the continental shelf regions, we compared atmospherically deposited DON_b to previously estimated total terrestrially derived N inputs (estuarine export and large river discharges directly to the shelf, plus atmospheric deposition directly to shelf waters; Nixon et al. 1996). In almost all of the coastal and shelf cases considered, the existing studies included only atmospheric DIN inputs. Therefore, to estimate the additional inputs from atmospheric DON, we multiplied the atmospheric DIN inputs by the appropriate amount based on the ratio of DIN to DON in atmospheric deposition reported for nearby locations. For the oceanic calculations, atmospheric N deposition (DON and DIN) and N_2 fixation are from Cornell et al. (1995). For all regions, bioavailable DON (DON_b) from atmospheric deposition was assumed equal to 45–75% of the atmospheric DON inputs, based on results of the current study. Additional data sources and details of the calculations are itemized in the Table 2 footnotes.

Across the six coastal systems, there is a wide range in the relative magnitude of estimated atmospheric inputs of DON_b or DIN plus DON_b compared to the sum of the N

Table 2. Estimated contribution of atmospheric deposition of bioavailable DON (DON_b) and total bioavailable N (DIN + DON_b) relative to N sources for various coastal, continental shelf, and oceanic regions.

	N inputs		Percent of N inputs due to atmospheric deposition of	
Region Coastal ecosystems ($\times 10^8$ mol N yr^{-1})	Sewage + agriculture	Atmospheric DIN + DON*	DON_b†	DIN + DON_b
Ochlockonee Bay (FL)‡§	0	0.17	22–38%	72–88%
Barnegat Bay (NJ)‖	0.1	0.55	7–12%	76–81%
Waquoit Bay (MA)¶	10.3×10^{-3}	9.9×10^{-3}	10–16%	37–44%
New York Bight‡#	73	62	6–10%	39–43%
Chesapeake Bay‡#	41	31	5–9%	36–39%
Narragansett Bay (RI)‡**	46	8	1–2%	12–13%
Continental shelf ($\times 10^{10}$ mol N yr^{-1})	Estuarine export††	Atmospheric DIN + DON‡‡	DON_b†	DIN + DON_b
North Atlantic§§				
45–60° western side of basin	2–5	2.5–5	3–32%	22–94%
eastern side of basin	5–9	7.5–15	4–34%	43–72%
20–45° western side of basin	15–20	5–10	2–18%	18–36%
eastern side of basin	0.5–1	0.25–0.5	2–23%	18–46%
Oceanic ($\times 10^{12}$ mol N y^{-1})‖‖‖‖	N_2 fixation	Atmospheric DIN + DON	DON_b†	DIN + DON_b
	1–3	4–11	9–50%	49–90%

* Includes atmospheric inputs directly to water surface as well as exported from watershed.

† Atmospheric deposition of bioavailable DON estimated equal to 45–75% of DON deposition (this study).

‡ Sewage, agriculture, and atmospheric DIN inputs from Hinga et al. (1991), which were based on N inputs to watershed and land use specific N retention coefficients.

§ Atmospheric DON assumed = 50% of TN based on 4-yr average annual data from Florida (Riekert 1983).

‖ Agricultural N and atmospheric N export from watershed calculated using approach of Hinga et al. 1991; watershed area = 1,554 km^2; watershed land use in 1987 from National Resources Inventory, Natural Resources Conservation Service, USDA; atmospheric N deposition = 123 mmol N m^{-2} yr^{-1} with 20% = DON from Seitzinger unpubl. data for New Brunswick, New Jersey; fertilizer use in watershed from Jordan pers. comm.

¶ Sewage, agriculture, and atmospheric DIN and DON deposition from Valiela et al. 1997; DIN and DON atmospheric export from watershed calculated from total atmospheric export from watershed (6,923 kg N yr^{-1} and average %DON in atmospheric deposition (43%); deposition on bay water surface calculated from average N deposition to watershed, watershed area (59 km^2), and bay surface area (630 ha; D'Avanzo et al. 1996).

Atmospheric DON estimated using average annual N composition (organic N = 28% of total N) of 14-yr data set from Chesapeake Bay region (Jordan et al. 1995).

** Atmospheric DON assumed = 19% of TN (Nixon et al. 1995).

†† Includes estuarine export plus large river discharges directly to shelf (Nixon et al. 1996); some undetermined portion of this originated as atmospheric deposition on the watershed.

‡‡ Atmospheric N deposition directly to surface of continental shelf waters; DIN summarized in Prospero et al. 1996 and Nixon et al. 1996; DON deposition assumed = 20–60% of total N in atmospheric deposition based on approximate range reported for annual studies in coastal watersheds in Atlantic Basin from Table 1.

§§ N inputs to shelf do not include across-slope inputs from oceanic sources.

‖‖‖‖ N_2 fixation and atmospheric DIN and DON deposition from Cornell et al. 1995; even if N_2 fixation is 14×10^{12} mol N yr^{-1} (highest estimates), atmospheric DIN + bioavailable DON still = 17–40% of N inputs. River N inputs are assumed to be removed by denitrification in continental shelf regions (Seitzinger and Giblin 1996) and thus are not included as a source of N to the open ocean.

inputs considered (sewage, agriculture, and total atmospheric N deposition) (Table 2). Atmospheric deposition of DON_b appears to be least important in Narragansett Bay, where it accounts for only about 1% of the N from sewage, agriculture, and the atmosphere. However, estimated inputs of atmospheric DON_b account for about 5–10% of the N inputs in Chesapeake Bay and the New York Bight, about 10–15% in Waquoit Bay and Barnegat Bay, and as much as 20–40% of the N inputs in Ochlockonee Bay. Obviously, DIN also is an important component of biologically available N deposited from the atmosphere. The sum of these two components (DIN + DON_b) can account for approximately 80–90% of the N inputs considered in Ochlockonee Bay and Barnegat Bay, only about 10% in Narragansett Bay, and approximately 40% in the other three coastal systems considered. The differences among these coastal systems in the relative contribution of atmospheric DON_b to estimated N inputs are due to a combination of factors for each watershed, including extent of development in the watershed, which contributes N inputs from sewage and agriculture, and percent contribution of DON to total N deposition. For example, in Ochlockonee Bay, 50% of the atmospheric N deposition is DON (Riekert 1983), and N inputs other than atmospheric are negligible, resulting in DON_b = ~20–40% of the N inputs. In Barnegat Bay, atmospheric N deposition is also the major N input; however, DON is only about 20% of the N deposition, resulting in about 10–15% of the N inputs attributable to atmospheric DON_b.

For continental shelf regions of the North Atlantic between 20 and 60°N, we estimate that atmospheric DON_b de-

posited directly to the surface of shelf waters could contribute <5% to as much as 20–30% of total terrestrially derived N inputs (Table 2). The sum of atmospheric DIN plus DON_b accounts for ca. 20 to >90% of the N inputs. For the global oceans, DON_b alone can account for ~10–50% of the N inputs from N_2 fixation and atmospheric deposition. While the above calculations include a large number of assumptions, they suggest that atmospheric DON_b is an important contributor to N loading in a wide range of marine ecosystems. In a number of cases, DON_b accounts for 10–40% of the N inputs. When combined with DIN, the role of the atmosphere as a biologically available N source is even greater; in most of the cases considered, it accounts for 20–90% of N inputs.

Increased N inputs to terrestrial and aquatic ecosystems result in a variety of environmental problems (Kemp et al. 1983; Rosenberg et al. 1990; Nixon 1992; Valiela et al. 1992). Given the ecosystem-level alterations associated with increases in N inputs and the potentially large flux of N to ecosystems from atmospheric deposition, a clear understanding of the sources, magnitude, chemical composition, and factors controlling atmospheric N deposition and their effects on receiving ecosystems will be critical in the coming decades. The present study suggests that a significant fraction of the organic N in atmospheric deposition is biologically available and furthermore, that this bioavailable DON can be an important component of N inputs compared to other sources to marine ecosystems.

S. P. Seitzinger

Rutgers University
Institute of Marine and Coastal Sciences
71 Dudley Road
Rutgers/NOAA CMER Program
New Brunswick, New Jersey 08901-8521

R. W. Sanders

Temple University
Department of Biology
Philadelphia, Pennsylvania 19122

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Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon

Abstract—It is generally accepted that marine bacteria utilize labile, recently produced components of bulk dissolved organic matter. This interpretation is based largely on indirect measurements using model compounds and plankton-derived organic matter. Here, we present an assessment of the relative proportions of modern and older dissolved organic carbon (DOC) utilized by marine bacteria. Bacterial nucleic acids were collected from both estuarine (Santa Rosa Sound, FL) and open-ocean (eastern North Pacific) sites, and the natural radiocarbon signatures of the nucleic acid carbon in both systems were determined. Bacterial nucleic acids from Santa Rosa Sound were significantly enriched in radiocarbon with respect to the bulk DOC and were similar to the radiocarbon signature of atmospheric CO_2 at the time of sampling, indicating that these bacteria exclusively assimilate a modern component of the estuarine bulk DOC. In contrast, bacterial nucleic acids from the oceanic site were enriched in ^{14}C relative to the bulk DOC but depleted in ^{14}C with respect to modern surface dissolved inorganic carbon (DIC) and suspended particulate organic carbon (POC_{susp}). This suggests that open-ocean bacteria assimilate both modern and older components of DOC. The distinct radiocarbon signatures of the nucleic acids at these two sites (i.e., $+120 \pm 17‰$ estuarine vs. $-34 \pm 24‰$ oceanic) demonstrate that natural ^{14}C abundance measurements of bacterial biomarkers are a powerful tool for investigations of carbon cycling through microbial communities in different aquatic systems.

Marine DOC represents one of the largest exchangeable reservoirs of organic carbon ($\sim 0.6 \times 10^{18}$ g C) at the earth's surface (Druffel et al. 1992; Hedges 1992). Heterotrophic bacteria are the primary consumers of marine DOC and influence its persistence in seawater through preferential utilization of specific components of the bulk DOC pool. However, despite extensive research examining bacterial DOC utilization in different marine systems (e.g., Rakestraw 1947; Barber 1968; Coffin et al. 1990; Kirchman et al. 1991; Peterson et al. 1994; Carlson and Ducklow 1996; Cherrier et al. 1996), there remains uncertainty in our current understanding as to the relative ages of the specific bulk DOC components supporting bacterial growth. On the one hand, short-term incubation studies (i.e., days to months) indicate that bacteria consume labile, recently produced DOC (Coffin et al. 1993; Carlson and Ducklow 1995, 1996; Cherrier et al. 1996; Coffin and Connolly 1997). On the other hand, as the concentration of oceanic bulk DOC is assumed to be at steady state (Norrman et al. 1995; Carlson and Ducklow 1996) and has an average conventional age of ~4,000–6,000 yr B.P. (Williams and Druffel 1987; Bauer et al. 1992; Druffel et al. 1992), it is generally thought that bacterial remineralization must also be one of the ultimate sinks for older, presumably more refractory bulk DOC constituents (Williams and Carlucci 1976). The latter, however, has yet to be directly demonstrated. The objective of this study was to examine and contrast the relative proportions of modern and older bulk DOC constituents assimilated by estuarine and oceanic bacteria using natural radiocarbon abundances of a specific biomarker, nucleic acids.

The carbon isotopic signatures of heterotrophic organisms, including bacteria, reflect the isotopic composition of the organic carbon sources they assimilate (Peterson and Fry 1987; Coffin et al. 1990). It is thus possible to determine what substrates are being consumed in situ by indigenous bacterial populations by measuring their isotopic compositions. Differential filtration cannot effectively isolate natural bacterial assemblages from inorganic and detrital particles of the same size (0.2–1.0-μm effective diameter) for whole-cell isotopic analysis (Coffin et al. 1989). An approach to separate bacteria from the detrital background is to isolate and analyze a biomarker that is specific to bacteria. Bacterial nucleic acids have been shown to have an isotopic composition similar to that of whole cells (Blair et al. 1985; Coffin et al. 1990; Kelly et al. 1998). The specificity of nucleic acids as a bacterial biomarker was confirmed by Coffin et al. (1990), who found that 95% of the 16S rRNA extracted from the 0.2- to 0.8-μm size fraction of estuarine water was bacterial. Comparison of the natural ^{14}C signatures of bacterial nucleic acids extracted from free-living bacteria with the ^{14}C signatures of bulk DOC constituents would additionally allow us to evaluate which age fractions of bulk DOC