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Rainfall stimulation of primary production in western Atlantic Ocean waters: roles of different nitrogen sources and co-limiting nutrients

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ABSTRACT: Using shipboard bioassays, we examined the roles rainfall, individual and combined nutrients play in accelerating primary production in coastal, Gulf Stream and pelagic (Sargasso Sea) locations in the North Atlantic Ocean off North Carolina, USA, from 1993 to 1995. Photosynthetic CO2 fixation and net chlorophyll a (chl a) production were measured in replicated bioassays to assess individual and combined impacts of different constituents of atmospheric deposition, including natural rainfall, a synthetic rain mix, dissolved inorganic nitrogen (DIN; NH4+, NO3-), dissolved organic nitrogen (DON; urea), phosphorus (PO43-) and iron (as EDTA-chelated and unchelated FeCl3). Natural rainfall and DIN additions most often stimulated CO2 fixation and chl a production, but frequencies and magnitudes of biostimulation, relative to controls, varied between these indicators. Spatial differences in the types and magnitudes of stimulation were also observed. When added in equimolar amounts, NH4+ was, at times, more stimulatory than NO3-. The NO3- stimulation was significantly enhanced by Fe-EDTA. Urea was marginally stimulatory at the coastal location. PO43- was never stimulatory. Fe-EDTA and EDTA by themselves stimulated production only at the offshore locations, suggesting increased Fe limitation with increasing distance from land. Synthetic rain, which contained both sources of DIN, but not Fe, generally proved less stimulatory per unit N than natural rainfall. Results indicate a broad sensitivity of these waters to N additions, which in the case of NO3- are enhanced by Fe-EDTA. At all locations, the high level of stimulation of primary production attributable to natural rain may be due to the supply of both DIN and co-limiting nutrients (e.g. Fe), contributing to the eutrophication potential of waters downwind of urban, industrial and agricultural emissions.

KEY WORDS: Atmospheric deposition - Nitrogen - Iron - Primary production - Eutrophication - W. Atlantic Ocean

INTRODUCTION

Nitrogen (N) supply frequently limits primary production in coastal waters (Dugdale 1967, Ryther & Dunstan 1971, Nixon 1986) and together with iron (Fe) plays an important role in controlling production in pelagic areas distant from land (Dugdale 1967, Martin et al. 1994). This common finding has fostered extensive research evaluating the roles that externally supplied, 'new' N inputs play in accelerating primary production and eutrophication of N-sensitive waters. One source of 'new' N is atmospheric deposition (AD) of anthropogenically produced N compounds, which has recently been recognized as a significant and growing fraction of external N loading (Paerl 1985, 1993, Duce 1986). Approximately 20 to 40% of 'new' N inputs into coastal waters downwind of continental regions are of atmospheric origin (Martin et al. 1989, Loye-Pilot et al.)

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1991, Prado-Fiedler 1990, Paerl 1993, 1995), much of it due to growing agricultural, urban and industrial emissions (Bribecome & Stedman 1982, Rodhe & Rood 1986, Buijsman et al. 1987, Galloway et al. 1994). The relative contribution of AD-N to coastal N budgets will increase substantially as we enter the next century, when nearly 70% of the European and North American populations will reside within 50 km of the coast (Galloway et al. 1994). On regional and global scales, AD-N is a significant contributor to oceanic 'new' N inputs, accounting for ~35 Tg N yr\(^{-1}\), compared to 30 Tg N yr\(^{-1}\) from riverine discharge, 5 to 10 Tg N yr\(^{-1}\) from groundwater and ~8 Tg N yr\(^{-1}\) from biological nitrogen fixation (Wollast 1991, Codispoti et al. in press). Atmospheric N is particularly important to surface seawater productivity because, unlike river discharge, AD-N does not pass through the 'estuarine filter' (Kennedy 1986), but rather is directly deposited on the sea surface.

Both wet and dry forms of AD contain various biologically reactive N compounds. In addition to the inorganic forms NH\(_4\)/NH\(_3\), NO\(_2\), NO\(_3\), there are organic N compounds such as urea, amino acids and organonitrites (Timperley et al. 1985, Duce 1986, Mopper & Zia 1987, Cornell et al. 1995, Peierls & Paerl 1997). Stimulation of marine primary production by these sources has been evaluated in bioassay-based studies spanning N-limited estuarine, coastal and oceanic ecosystems (Thayer 1974, Paerl et al. 1990, Willey & Cahoon 1991, Willey & Paerl 1993, Paerl & Fogel 1994, Peierls & Paerl 1997). While dissolved inorganic N (DIN) compounds in rainfall have been clearly shown to stimulate primary production (Paerl 1985, Paerl et al. 1990), these studies also suggested that some factor(s) other than DIN play a biostimulatory role. For example, Paerl et al. (1990) and Paerl & Fogel (1994) noted that, per amount of N, rainfall produced more stimulation than either NH\(_4\) or NO\(_3\) additions.

Suggested potential 'missing factors' include nutrient trace metals and dissolved organic N (DON) (Paerl & Fogel 1994). Recently, Peierls & Paerl (1997) examined the bioreactivity of DON fractions in rainfall. DON proved stimulatory to nearshore phytoplankton, but at magnitudes far less than DIN. Moreover, DON compounds previously identified in rainfall (urea, amino acids) failed to enhance production of offshore oligotrophic phytoplankton communities. Therefore, factors in addition to DIN and DON may cause the potential fertilizing impact of rainfall, and the role of aerosol trace nutrients is considered here.

Continental dust (e.g. Saharan soil dust, volcanic, urban and industrial particulate emissions) can be deposited on the sea surface as dry deposition, or incorporated into rain by washout and deposited as wet deposition (Church et al. 1984, Duce & Tindale 1991). The amount of soil dust in the atmosphere may increase as a result of expanded agricultural practices, which would increase crustal elements in AD, including Al, Fe, Si and P (Duce et al. 1991). Certain crustal elements including Fe may increase in solubility in aerosols as a result of photooxidation (Behra & Sigg 1990, Zhuang et al. 1992, 1995) and repeated exposure to acidic cloudwater (Spokes et al. 1984, Jickells 1995). Aerosols from anthropogenic sources, including combustion processes, may also increase as a result of industrial growth. Trace metals, including Cd, Pb, Zn, Cu, Ni and V, are thought to be elevated in rain relative to crustal concentrations (Church et al. 1984). Rain is thus a delivery mechanism for many trace metals, and provides a solubility-enhancing environment for some of them (Duce & Tindale 1991, Duce et al. 1991).

Among nutrient trace metals in continentally derived AD, Fe has been shown to be a stimulant of marine primary production (Martin et al. 1994). AD is the primary source of new iron to the oceans (Duce 1986, Duce & Tindale 1991), and Fe has been shown to enhance marine primary production in geographically diverse oceanic waters (Martin et al. 1991, 1994, DiTulio et al. 1993, Takeda et al. 1995). Stimulation was maximal in the presence of N (as NO\(_3\)) enrichment. Likewise, Paerl et al. (1994) demonstrated that growth and N\(_2\) fixation of the bloom-forming planktonic cyanobacterium Trichodesmium spp. were enhanced by Fe additions in N-depleted coastal and nearshore Atlantic Ocean (Gulf Stream) waters.

The enzymes responsible for NO\(_3\) reduction to NH\(_3\) (nitrite and nitrate reductases) and N\(_2\) fixation (nitrogenase) contain Fe (Stewart 1974). Therefore, phytoplankton relying on these new N compounds have relatively high requirements for this metal, and under conditions of NO\(_3\) enrichment Fe requirements for synthesis of these enzymes are high. Continually derived rainfall and dryfall are enriched with N (DIN = 20 to >50 \(\mu\)M, mostly as NO\(_3\); DON = 5 to 20 \(\mu\)M), and N enrichment from this source may increase the potential for Fe limitation, which in turn could control new production, f-ratios and phytoplankton community composition (Harrison et al. 1987). This scenario is most applicable to pelagic waters, where iron availability is often severely restricted and aeolian inputs are the sole source of new Fe (Martin et al. 1994).

In eastern North Carolina, riverine inputs are small, and nutrients are effectively assimilated by estuarine and coastal surface phytoplankton communities. This leads to relatively low levels of 'new' nutrient discharge into coastal and oceanic waters by rivers (Copeland & Gray 1991), and suggests that atmospheric and advective (upwelling/deep mixing) nutrient inputs may be key sources of 'new' nutrients supporting primary production. The proximity and
predominantly downwind location of oligotrophic Gulf Stream and Sargasso Sea surface waters to the North American continent further suggest that atmospheric deposition may be very important as a source of multiple, synergistically interacting nutrients to this oceanic region. In the present paper we investigate the possibility that Fe may play a key synergistic role in the observed potential (based on N alone) fertilizing capability of AD.

**MATERIALS AND METHODS**

**Sampling locations.** The impacts of rainfall were compared with individual and combined N and Fe enrichments in North Carolina nearshore and offshore western North Atlantic waters previously documented as being N limited (Paerl 1985, Paerl et al. 1990). Transects (~200 km) of nearshore to offshore locations were sampled at 3 locations during 5 cruises of the RV 'Cape Hatteras' from 1993 to 1995. The nearshore (inner continental shelf) location was approximately 25 km SE of Beaufort, North Carolina (Fig. 1); an offshore location was near the western boundary of the Gulf Stream, ranging from 60 to 100 km offshore. A third oceanic station was located well beyond the eastern boundary of the Gulf Stream in the Sargasso Sea, approximately 150 to 200 km SE of Beaufort. These locations were sampled on 5 occasions between 1993 and 1995, during late spring (May to June) and fall (September to November).

**Experimental procedures.** Seawater was collected at 1.5 m depth with a non-metallic (PVC-lined) diaphragm pump attached to natural rubber hoses, which were flushed for several hours prior to dispensing seawater into polyethylene Cubitainers for nutrient addition bioassays. The Cubitainers were first rinsed with 1% HCl, followed by exhaustive seawater (from each sampling location) rinses prior to filling. Both 1 and 4 l Cubitainers were used as bioassay vessels, the former for 14C-based measurements of photosynthesis and the latter for chlorophyll a (chl a)-based determinations of biomass. Cubitainers are chemically inert and approximately 80% transparent to photosynthetically active radiation (PAR: 400 to 700 nm) (Paerl et al. 1990). Each 1 l Cubitainer was supplied with 900 ml seawater including nutrient or rain additions, while the 4 l vessels contained 3.5 l seawater. Six unamended controls were run in parallel with treatments. Experimental treatments were run in triplicate. We examined impacts of nutrients previously implicated in the control of primary production in these waters. These included: DIN (NH4+, NO3-), DON (urea), phosphorus (PO43-) and Fe (equimolar EDTA-chelated and unchelated FeCl3). Prior work (Paerl et al. 1990, Rudek et al. 1991) indicated silicon (Si) sufficiency under a range of N, P and trace metal enrichment conditions. Treatments and nutrient additions are shown in Table 1.

All nutrient and rain additions were dispensed from polyethylene (Nalgene) bottles. Fe and EDTA solutions were freshly mixed and stored in Teflon containers and dispensed with polyethylene pipette tips cleaned with 1% HCl and 18 MΩ deionized water. Reagents were analytical grade. In the case of Fe salts (Fisher), ultrapure reagent grades were used. The EDTA used was supplied and shown to be essentially free of Fe by Dr K. Bruland, University of California, Santa Cruz. Efforts were made to ensure 'clean techniques' throughout bioassay procedures.

Rainwater was collected with precleaned polyethylene funnels and carboys on the laboratory rooftop (Morehead City, North Carolina, USA) during storms prior to the bioassays. Precleaning included a 1% HCl rinse followed by several rinses of 18 MΩ deionized water. Nutrient (NO3-/NO2-, NH4+, organic N, PO43-) content of the rainwater was determined. If not used immediately, rainwater was stored frozen (~20°C) in carboys.
and reanalyzed for nutrient concentrations shortly before use in bioassays. During 1994, we encountered an event of heavy rainfall containing 125 μM NH₄⁺. This event, termed 'high NH₄⁺', was used in the May 1994 bioassays. The final DIN (NH₄⁺ + NO₂⁻/NO₃⁻) concentration after natural rain additions ranged from 2.1 to 3.1 μM N respectively, while the high NH₄⁺ natural rain addition resulted in a final concentration of 4.8 μM N.

The synthetic rainwater composition was 20 μM HNO₃, 5 μM NH₄⁺ as (NH₄)₂SO₄, and 20 μM H₂SO₄ in 18 MΩ deionized water (pH = 4.25). The composition of the synthetic rain was checked for pH by low-conductivity probes and by ion chromatography before and after each bioassay.

Seawater, rainwater and nutrients were added to Cubitainers with Nalgene polyethylene graduated cylinders or autopipetted using precleaned polyethylene or polypropylene tips. The 1 l Cubitainers received 7.5 μCi of ¹⁴C-Na bicarbonate (60 mCi mmol⁻¹; ICN Inc.) for determining photosynthetic CO₂ fixation. Following additions, Cubitainers were sealed and transported to on-deck plastic wading pools filled with circulating seawater, exposed to natural irradiance and temperatures. Previous time-course studies using Cubitainers (Paerl et al. 1990, Rudek et al. 1991) indicated that for these waters optimal growth yields were obtained within 2 to 3 d following nutrient additions. Longer incubation periods could lead to methodological artifacts, including fouling of the vessels, dissolved inorganic carbon depletion and grazing impacts. On sunny days, 1 layer of neutral density screening (reducing incident irradiance by 30%) was placed over pools to minimize photoinhibition and photooxidation of natural phytoplankton communities. The ship's motion ensured continual mixing of cubitainers.

For ¹⁴C fixation measurements, the entire 900 ml in 1 l Cubitainers was vacuum filtered (200 mm Hg) through 25 mm Whatman GF/F filters (approximate pore size = 0.4 μm). Filters were air dried, fumed with HCl vapors for 2 h, air dried again, placed in 7 ml of Ecolume cocktail (ICN Inc.) and analyzed by liquid scintillation spectrometry (Beckman LS 7500) (Paerl 1987). The entire 3.5 l in each large Cubitainer was filtered onto a 4.8 cm GF/F filter which was subsequently analyzed for chl a by fluorometry using a Turner Model 110 fluorometer (Strickland & Parsons 1972). Initial dissolved inorganic N and P concentrations were determined on GF/F filtered and frozen subsamples from all waters assayed. Nutrients were subsequently analyzed using a high sensitivity automated analyzer following the manufacturer's instructions (Lachat Quick Chem. IV, Lachat Instruments, Milwaukee, Wisconsin, USA; NH₄⁺, Method 31-107-06-1-A; NO₂⁻/NO₃⁻, 31-107-04-1-C; PO₄³⁻, 31-115-01-3-A).

Rainwater was not analyzed for Fe content because of difficulties in ensuring trace metal free conditions in such determinations. Instead, we referred to the rain iron concentration data of Church et al. (1984) from Lewes, Delaware, USA (0.27 μM) and Bermuda (0.09 μM), and from Church et al. (1991) for oceanic rain (from 0.1 to 0.5 μM) to plan our experiments. Fe concentrations of ambient surface seawater were not measured; concentrations were most likely in the nanomolar to subnanomolar range (Povell et al. 1995, Zhuang et al. 1995). The amount of iron added was chosen to give an excess of at least 10 times ambient quantities. This concentration is below the solubility of iron in the presence of EDTA in seawater. Rainwater analyses of dissolved organic carbon (DOC) (University of North Carolina at Wilmington) during summer 1996 indicate that DOC varied from 30 to 500 μM, with an average of approximately 300 μM. This suggests that at least some of the iron in rain may be organically complexed, which makes addition of iron as the EDTA complex more realistic.

All bioassay responses were examined relative to the control. Productivity responses of the phytoplankton community measured as ¹⁴CO₂ fixation or chl a content were compared statistically for each bioassay using an a posteriori comparison of means procedure (Bonferroni, p < 0.05). Values were natural log (ln) transformed before analysis to normalize the data.

RESULTS

Ambient nutrients

Ambient nutrient concentrations at all locations and all dates proved to be uniformly low and near the limit of detection. For NO₂⁻/NO₃⁻-N, ambient concentrations were between 0.02 and 0.3 μM, with springtime (May 1994, May 1995) concentrations tending to be

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final concentrations/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No rain/nutrients added</td>
</tr>
<tr>
<td>Natural rain</td>
<td>1-3% v/v additions, 3% in most bioassays</td>
</tr>
<tr>
<td>Synthetic rain</td>
<td>1-3% v/v additions, 3% in most bioassays</td>
</tr>
<tr>
<td>NO₃⁻-N (as NaNO₃)</td>
<td>5 μM</td>
</tr>
<tr>
<td>NH₄⁺-N (as NH₄Cl)</td>
<td>5 μM</td>
</tr>
<tr>
<td>Urea-N</td>
<td>5 μM</td>
</tr>
<tr>
<td>PO₄³⁻-P (as Na₂HPO₄)</td>
<td>2 μM</td>
</tr>
<tr>
<td>Fe²⁺ (as FeCl₃)</td>
<td>0.05-1 μM</td>
</tr>
<tr>
<td>EDTA (as Na₂EDTA)</td>
<td>0.05-1 μM</td>
</tr>
<tr>
<td>FeCl₃ + EDTA</td>
<td>0.2 + 0.2 μM</td>
</tr>
<tr>
<td>FeCl₃ + EDTA + NO₃⁻</td>
<td>0.2 + 0.2 + 5 μM</td>
</tr>
</tbody>
</table>

Table 1 Rainwater and nutrient additions in Cubitainer bioassays.
higher. The one exception followed Hurricane Gordon
( November 1994), when extensive vertical mixing
introduced 0.5–0.9 μM to surface waters. Ammonium
(NH₄⁺-N) concentrations were consistently <0.2 μM on
all sampling dates at all locations. After the passage of
Hurricane Gordon NH₄⁺ levels were slightly elevated
(0.4 to 0.6 μM) in the upper water column at all three
locations. The DIN enrichment resulting from Hurri-
cane Gordon caused increases in both primary produc-
tion and chl a concentrations of impacted waters
(Fogel et al. in press). Orthophosphate (PO₄³⁻-P) con-
centration ranged from 0.2 to 0.7 μM at all locations
and dates, with slightly higher values (0.5 to 1.2 μM)
following Hurricane Gordon.

Bioassays

Among bioassay treatments, individual N and nat-
ural and synthetic rainfall additions proved most stim-
ulatory to CO₂ fixation and chl a production. Substan-
tial variation in the frequencies and magnitudes of
stimulation, relative to controls, was observed among
sampling locations throughout the sampling period.
On occasions, natural rainfall proved more stimulatory
than synthetic rain (Table 2). When rainfall contained
relatively high NH₄⁺ concentrations (4.8 μM N final
concentration in May 1994), both CO₂ fixation and
chl a showed the highest magnitudes of stimulation,
which were most significant at the inner shelf location
(Table 2, Fig. 2). While rainfall additions did not yield
statistically significant impacts on production at the
Gulf Stream and Sargasso Sea locations during 1994
(due to large standard deviation values relative to
absolute ¹⁴CO₂ fixation and chl a values among repli-
cates at these locations), stimulation relative to controls
was routinely observed during the experiments con-
ducted in the present study. When taking all locations
and dates into consideration, natural rainfall stimu-
lated production from 136 to over 400%, while syn-

<table>
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<tr>
<th>Treatment</th>
<th>Nov 1993 Chl a</th>
<th>Nov 1994 Chl a</th>
<th>May 1994 Chl a</th>
<th>May 1995 Chl a</th>
<th>Sep 1995 Chl a</th>
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<tbody>
<tr>
<td>Inner shelf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic rain</td>
<td>3%</td>
<td>ns</td>
<td>+211</td>
<td>+247</td>
<td>+124</td>
</tr>
<tr>
<td>Natural rain</td>
<td>3%</td>
<td>+182</td>
<td>+239</td>
<td>+260</td>
<td>+136</td>
</tr>
<tr>
<td>Nitrate</td>
<td>5 μM</td>
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<td>+360</td>
<td>+310</td>
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<tr>
<td>Ammonium</td>
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</tr>
<tr>
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<td>+360</td>
<td>+300</td>
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<tr>
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<td>ns</td>
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<td>+475</td>
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<tr>
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<td>+360</td>
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<tr>
<td>FeCl₃ + EDTA</td>
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<td>+400</td>
<td>+475</td>
<td>+321</td>
</tr>
<tr>
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<td>+169 +162</td>
<td>ns</td>
<td>+310</td>
<td>+360</td>
<td>+300</td>
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<tr>
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<td>+321</td>
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<td>Gulf Stream</td>
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<td>+400</td>
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<td>+321</td>
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<td>ns</td>
<td>-nd</td>
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Fig. 2 Bioassays of productivity responses, shown as both $^{14}$CO$_2$ fixation (disintegrations per minute, DPM, upper panel) and chl a concentrations (lower panel), of coastal Atlantic Ocean phytoplankton assemblages to a variety of rainfall and nutrient additions in May 1994. Nutrient additions are shown in Table 1. Final DIN ($NH_4^+ + NO_2^- / NO_3^-$) concentration in the natural rain addition was 2.8 µM N, while the high $NH_4^+$ natural rain addition resulted in a final concentration of 4.8 µM N. Treatments are plotted relative to the control, with ±1 SD shown. Treatments that were significantly different from controls are indicated by an asterisk. ‘Fe’ indicates unchelated $FeCl_3$. Bioassays were incubated for 2 d.

Fig. 3 Phytoplankton $^{14}$CO$_2$ fixation responses to nutrient and synthetic and natural rain additions at (a) inner shelf, (b) Gulf Stream and (c) Sargasso Sea locations during November 1993. The same natural rain event (2.1 µM final DIN concentration) was added at all locations. Bioassays were incubated for 2 d.

No strong seasonal (i.e. spring vs fall within any single year) trends were observed at any of the locations for either $^{14}$CO$_2$ fixation or chl a. The magnitudes of rainfall-based DIN stimulation varied spatially and temporally. Some of the observed variation may be attributed to differential DIN or DON content of either the rainfall or receiving waters and contrasting phytoplankton assemblages in diverse water masses during the experimental period (cf. Paerl 1985, Paerl et al. 1990, Paerl & Fogel 1994, Peierls & Paerl 1997). At times, natural and synthetic rain additions also led to different magnitudes of stimulation, despite yielding comparable final DIN levels (i.e. ambient + added DIN, Figs. 2, 3 & 4), suggesting that factors other than just DIN content played a role in the observed biostimulation. In 40% of all bioassays, NH$_4^+$ and NO$_3^-$ both significantly stimulated production, when added as similar amounts of N (Table 2). At times, however, NH$_4^+$ enrichment led to greater and more significant stimulation than NO$_3^-$; this was most noticeable at the Gulf Stream location where significant differences were especially evident in $^{14}$CO$_2$ fixation bioassays conducted in May and September, 1995 (Table 2).

FeCl$_3$ alone generally failed to significantly stimulate either $^{14}$CO$_2$ fixation or chl a (Figs. 2, 3 & 4, Table 2). In contrast, EDTA and FeCl$_3$ + EDTA often stimulated
productivity, especially at the more offshore Gulf Stream and Sargasso Sea locations (Table 2). When combined, FeCl₃ + EDTA + NO₃⁻ often exceeded stimulation observed by either FeCl₃ + EDTA or NO₃⁻ alone (Fig. 5). Overall, FeCl₃ + EDTA together with NO₃ stimulated productivity (as either ¹⁴C fixation or chl a) in 12 of the 13 bioassays (Table 2). Synergistic Fe-NO₃⁻ stimulation was most significant at the offshore locations throughout the sampling period (Table 2).

Urea additions proved significantly stimulatory in 3 of 12 bioassays (May 1994 and September 1995) at the inner shelf location, while offshore (Gulf Stream) stimulation was noted only once during September 1995 (Table 2).

Phosphate additions were not significantly stimulatory (Table 2, Figs. 2–5). This indicated that P supplies were adequate at all locations at any time, confirming previous nutrient limitation studies in these waters (Thayer 1974, Paerl 1985, Paerl et al. 1990, Rudek et al. 1991).

**DISCUSSION**

In this study, we evaluated parallel responses of both photosynthetic CO₂ fixation and phytoplankton chl a content to rainfall additions and to individual and combined nutrient enrichment. This enabled us to examine and evaluate the degree of agreement among these commonly used individual indicators of phytoplankton community responses to nutrient enrichment in space and time. ¹⁴C fixation and chl a showed similar, but not identical, responses to nutrient and rainfall additions. There were notable discrepancies in terms of relative degrees and total magnitudes of stimulation and differences (based on statistical significance) between treatments and controls as well as among treatments. Both parameters are indicative of phytoplankton production responses; however, they reflect and integrate different physiological, biosynthetic and community structuring processes. The ¹⁴C method measures cumulative net incorporation of ¹⁴CO₂ into particulate matter, initially mediated by photosynthesis. Net incorporation integrates photosynthetic activity, respiration, heterotrophic decomposition and graz-

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Fig. 4. Phytoplankton ¹⁴C fixation (upper panel) and chl a responses (lower panel) to nutrient, synthetic and natural rain additions at the Gulf Stream location in May 1994. The final DIN concentration after natural rain addition was 2.8 μM, while the high NH₄⁺ addition yielded 4.5 μM N. Bioassays were incubated for 2 d.

Fig. 5. Phytoplankton ¹⁴C fixation responses to nutrient additions in Gulf Stream (upper panel) and inner shelf (lower panel) Atlantic Ocean waters during September 1995. All bioassays were incubated for 3 d.
ing over the time of incubation. Earlier time-course bioassays (cf. Paerl et al. 1990, Rudek et al. 1991) showed variation in time-course slopes of net CO₂ fixation within a 2 to 5 d incubation period, possibly indicative of differential interactions among these processes. The chl a method is an integrated measure of chl a synthesis versus degradation (death) and grazing. In addition, different types and amounts of nutrient enrichment are known to have a selective effect on phytoplankton community size and composition (Stolte et al. 1994), which in turn could lead to diverging rates of ¹⁴C fixation and chl a synthesis. While this possibility was not investigated here, it has recently been verified for nearby North Carolina estuarine waters (Pinckney et al. 1998). We also observed differences in the degree to which a treatment differed from the control over time. For example, while the ¹⁴C method always showed net ¹⁴C incorporation during the incubation period for both controls and treatments, this was not always the case for chl a. Declines in chl a between the time of initiation and sampling of the bioassay could have been due to grazing, heterotrophic decomposition, altered turbulence and light regimes (cf. Paerl et al. 1990, Rudek et al. 1991, Willey & Paerl 1993).

Although N limitation was evident in near- and offshore waters, the forms in which N was added as well as the medium in which it was administered (e.g. defined DIN sources, natural and synthetic rain) modulated the productivity response. The fact that NH₄⁺ was, at times, more stimulatory than NO₃⁻ per amount of N administered suggests that NO₃⁻ reduction (i.e. nitrate reductase activity) was an important 'limiting' step controlling N assimilation and resultant growth stimulation of the phytoplankton community. Since Fe-EDTA additions enhanced the biostimulation observed with NO₃⁻ (relative to NH₄⁺), we suspect that, when NO₃⁻ enrichment occurred, Fe availability may have controlled the magnitude of productivity stimulation.

Rain high in NH₄⁺ relative to NO₃⁻ was particularly effective at stimulating productivity (Figs. 2 & 4). This may be related to our observation that NH₄⁺ additions are capable of stimulating productivity independent of the Fe status of rain and receiving waters (i.e. since Fe is not required for assimilation of NH₄⁺). On an annual basis, rainfall DIN at either Morehead City or Wilmington, NC, is composed of approximately 65% NO₃⁻ and 35% NH₄⁺ (Willey et al. 1988, Paerl & Fogel 1994). The dominant form of DIN deposited in adjacent Atlantic coastal waters may therefore be dependent on Fe availability in order to optimally support 'new' production.

In previous experimental work (Paerl et al. 1994) in these waters, EDTA (same source as used here) by itself frequently led to biostimulation, while Fe-EDTA led to the highest degree of stimulation (Paerl et al. 1994). This effect was also observed here, being most pronounced at the offshore Gulf Stream and Sargasso Sea locations (Table 2, Fig. 5).

Alternative explanations for these observations exist. EDTA may be enhancing nutrient trace metal availability by maintaining these metals in a dissolved form, counteracting potential losses from the water column by scavenging or precipitation. Since the enhancement was most pronounced in offshore waters, it would suggest that constraints on trace metal, specifically Fe, availability are most severe in these waters. Because no large rivers discharge into this region, land-based inputs of Fe and potential natural organic chelators (e.g. humic and fulvic substances) ensuring Fe sufficiency are confined to the coastal inner shelf waters. As a result, EDTA and FeCl₃ + EDTA additions frequently failed to lead to significant stimulation of production in these waters, while they did lead to stimulation in Fe- and/or chelator-devoid offshore waters. In addition, it has been suggested that EDTA stimulates productivity by chelating a potentially toxic metal (i.e. Cu) and thereby minimizing its exposure to phytoplankton (Bruland et al. 1991). We previously (Paerl et al. 1994) tested this possibility by adding relatively large amounts (0.1 μM of Cu (as CuSO₄) in the presence and absence of EDTA and examining productivity responses. No significant EDTA effect was observed in either coastal or offshore waters, casting doubt on this possibility. We therefore suggest that EDTA acts as a true biostimulant, most likely by enhancing nutrient trace metal availability.

Trace metal determinations of oceanic rainfall suggest an approximately 2-fold enrichment of Fe relative to crustal Al (Church et al. 1991). A large fraction (>80%) of the NO₃⁻ present in AD can be traced to human activities, including fossil fuel combustion and biomass burning (Briblecome & Stedman 1982, Galloyow et al. 1994). These 2 nutrients are delivered together to surface seawater by AD, and may at times function synergistically, stimulating primary production far more in combination than alone. The observed co-stimulatory impacts of NO₃⁻ and Fe and possibly other metals in AD (Duce et al. 1991) have biogeochemical and trophic ramifications. A growing number of coastal and pelagic waters experiencing accelerating eutrophication are downwind of major anthropogenic sources of AD-N (Paerl 1988, 1995, 1997). These include (but are not limited to) the Baltic and North Seas (Boalch 1987, Smetacek et al. 1991, Riegman et al. 1992), the western Mediterranean Sea (Martin et al. 1989, Loye-Pilot et al. 1990), the western North Atlantic seaboard (Anderson 1989, 1995), the Yellow Sea (Zhang 1994) and the Sea of Japan (Hallegraeff 1993). With few exceptions, these waters have exhibited symptoms of eutrophication, including in-

Increasing anthropogenic nutrient impacts on coastal zone (and beyond) trophodynamics dictate the need for a detailed, comprehensive view of the composition and nature of accelerated nutrient loading as it pertains to chronic and acute biogeochemical and trophic impacts. While we have recognized enhanced N loading as a key factor in coastal eutrophication, co-stimulatory interactions with other nutrients may modulate these impacts, thus altering production and food web dynamics. Iron has been implicated as such a modulator. Other trace metals (e.g. Cu, Zn, Mn, Pb, Hg, Mo, etc.) as well as organic N compounds resulting from industry, agriculture and urbanization require close scrutiny, for they could play additional, yet to be discovered roles in this regional and global process.

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LITERATURE CITED


Copeland BJ, Gray J (1991) Status and trends report of the Albemarle-Pamlico estuarine study. NC Dept Nat Resources & Community Dev Public, 90–01, Raleigh, NC


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