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# High rates of ammonium recycling drive phytoplankton productivity in the offshore Mississippi River plume

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ABSTRACT: As part of an integrated study of the regulation of carbon fixation in the offshore Mississippi River plume, we measured the rates of $^{15}$N-labeled ammonium and nitrate uptake in the surface plume waters from offshore to nearshore along the plume axis towards the Mississippi Delta. Concentrations of nitrate in the plume ranged from 0.19 to 2.5 µM with the highest concentrations primarily in the shoreward stations, while ammonium ranged from 0.17 to 0.44 µM, showing little spatial variability. Rates of ammonium uptake ranged from 16.5 to 260 nM $h^{-1}$, and showed a strong trend of increasing values from offshore towards the Mississippi Delta. In contrast, nitrate uptake rates ranged from 3.2 to 25 nM $h^{-1}$. The high rates of ammonium uptake in the presence of low ammonium concentrations and elevated nitrate was made possible by elevated rates of ammonium regeneration that exceeded ammonium uptake by 1.7 to 5.7-fold in the plume. The plume exhibited relatively low *f*-ratios and also contained elevated levels of *Synechococcus* as determined by flow cytometry and high levels of form IA (α-cyanobacterial) *rbcL* transcripts. These data suggest that a major portion of the carbon fixation observed in the offshore Mississippi River plume represents recycled production supported by high rates of ammonium regeneration.

KEY WORDS: Gulf of Mexico · Mississippi River plume · Nitrate uptake · Ammonium uptake · Nutrient cycling



## INTRODUCTION

High chlorophyll surface plumes originating from the world's major river deltas are often seen as prominent features in ocean color satellite images. Such plumes can extend far into the open ocean. For example, the Amazon and the Orinoco river plumes have been observed to extend as much as 1000 km into the western oligotrophic Atlantic Ocean and Caribbean Sea, respectively (Müller-Karger et al. 1989, 1995, Longhurst 1993).

The prominent river plume in the Gulf of Mexico (GOM) originates from the Mississippi River. The Mississippi discharges ca. 536 ± 130 $km^3$ of nutrient-rich water onto the northern GOM shelf each year, making it the 6th largest river worldwide (Dai & Trenberth 2002). Exacerbated by heavy fertilization of large areas within the Mississippi watershed (>2 million t of nitrogen fertilizer $yr^{-1}$) the river carries a very high nitrate (111 ± 4.3 µM) and phosphate (7 ± 0.4 µM) load as it reaches the delta (Amon & Benner 1997). During most of the year, this leads to massive phytoplankton blooms along the Louisiana and Texas coastlines, which receive most of the Mississippi plume freshwater input. In some instances, however, particularly during the summer months when local wind forcing and surface circulation are favorable, the Mississippi River plume instead reaches hundreds of kilometers into the eastern oligotrophic Gulf of Mexico. Under these circumstances ocean color images have shown the Mississippi plume to extend along the Florida shelf break as far as the Dry Tortugas or even the Florida Straits (Müller-Karger et al. 1991, Wawrik et al. 2003).

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No targeted survey of the plume in this environment has thus far been conducted to study the plume's effect on oligotrophic ocean ecology, phytoplankton composition and nutrient cycling. Previous studies of the plume have primarily been concerned with the plume's impact on coastal ecosystems in the northern GOM shelf region. The plume has, however, been shown to greatly enhance oligotrophic surface productivity and phytoplankton species composition in the oligotrophic GOM (Wawrik et al. 2003).

On the shelf, nutrients, irradiance and primary production of the Mississippi River plume have been studied extensively (Lohrenz et al. 1990, 1994, 1999, Dortch & Whitledge 1992, Pakulski et al. 1995, 2000). Productivity in the most coastal region of the plume is initially limited by turbidity, and highest productivity occurs at intermediate salinities as the plume matures. Nutrients display distinct non-conservative mixing behavior along the salinity gradient of the plume, and both silicate as well as nitrogen have been reported to limit productivity in the higher salinity portions of the plume. High productivity in the plume has been implicated in the formation of extensive regions of hypoxia in bottom waters along the Louisiana and Texas shelf during summer stratification (Dortch et al. 1994, Eadie et al. 1994, Rabalais et al. 1994, 1996, Justic et al. 1996).

Nitrogen mineralization rates in the Mississippi River plume area have been measured using High Performance Liquid Chromatography (HPLC) in isotope dilution and enrichment experiments (Gardner et al. 1993). It was found that highest ammonium regeneration rates occurred in samples from shallow depths where primary and bacterial production was high. In the Mississippi plume region, ammonium regeneration rates, bacterial production, and amino acid turnover have been observed to be greatest at intermediate salinities during the summer (Cotner & Gardner 1993).

In order to determine the effect of the offshore Mississippi River plume on nitrogen cycling in the oligotrophic Gulf of Mexico, we measured the concentrations and relative uptake rates of dissolved inorganic nitrogen (DIN) species along a transect of the plume from offshore to onshore. Our results indicate that nitrate concentrations averaged over 9-fold higher than ammonium concentrations in the plume, yet uptake rates of ammonium were almost 7-fold greater than nitrate uptake rates. Recycled production thus dominated within the plume, an observation that was corroborated by the presence of a numerically dominant population of *Synechococcus*. In a companion study, we explore the importance of the offshore plume to oligotrophic productivity in the Gulf and describe phytoplankton species dynamics using molecular techniques and pigment analysis (Wawrik & Paul 2004, this issue).

## MATERIALS AND METHODS

**Gulf of Mexico sampling.** Surface samples were obtained aboard the RV 'F. G. Walton-Smith' between July 16 and 26, 2001, along the approximate axis of the offshore Mississippi River plume. For a more detailed description of our sampling strategy as well as a color Seaviewing Wide Field-of-view Sensor (SeaWiFS) satellite image of the sampling region, please refer to Wawrik & Paul (2004).

**Ambient nutrient concentrations.** Water from each depth of the vertical profile was filtered through precombusted (450°C for 2 h) Whatman GF/F filters. Concentrations of nitrate, phosphate and silicate were measured with a Technicon AutoAnalyzer. Concentrations of $NH_4^+$ were measured manually with the phenol/hypochlorite technique (Grasshoff et al. 1999).

**Uptake and regeneration of inorganic nitrogen.** Rates of ammonium and nitrate uptake were measured in duplicate with $^{15}N$ tracer techniques using 0.05 µM tracer additions as previously described (Bronk et al. 1998). All $^{15}N$ tracer incubations were done in on-deck flow-through incubators under simulated *in situ* light and temperature conditions. Light was attenuated with blue Plexiglas shields and neutral density screens. Experiments were done in 1 l polyethylene terephthalate copolyester, glycol modified (PETG) bottles, and samples were incubated for ~3 h. At the end of each incubation, samples were filtered through precombusted (450°C for 2 h) GF/F filters. Filters were subsequently dried and analyzed on a Europa GEO20/20 mass spectrometer with an automated nitrogen carbon analyser (ANCA) sample processing unit. The filtrates from the ammonium incubations were collected and frozen for later determination of the atom% enrichment of the $NH_4^+$ pool. The ammonium pool was isolated using the solid phase extraction technique (Dudek et al. 1986, Brzeninski 1987). All $NH_4^+$ uptake rates were corrected for isotope dilution (Glibert et al. 1982).

**$^{14}$C-carbon fixation.** Photosynthetic carbon fixation was measured as a modification of the methods of Carpenter & Lively (1980) as modified in Wawrik et al. (2003) and described in Wawrik & Paul (2004).

***rbcL* mRNA analysis.** mRNA was extracted from seawater using RNeasy spin columns (Qiagen) as described elsewhere (Paul 2001). Briefly, between 200 and 800 ml seawater samples were treated with 0.1% v/v DEPC (diethyl-pyrocarbonate; Sigma Chemical) and filtered onto 25 mm, 0.45 µm HV polyvinylidene difluoride filters (Millipore Durapore). Filters were stored in liquid nitrogen in 750 µl of RLT lysis buffer (Qiagen) together with 0.2 g of baked, muffled glass beads (Biospec Products). Cell lysis was achieved by bead-beating. The lysate was then extracted following the RNeasy kit (Qiagen) protocol. Samples were split 3 ways. One-third

was digested with DNAse-free RNAse and one-third was digested with RQ1-DNAse. RNA was then immobilized onto Zeta-Probe charged nylon filters (Bio-Rad) by dot-blotting and UV-crosslinking. Duplicate samples were probed with form IA, form IB and form ID *rbcL* probes as previously described (Watson & Tabita 1996, Paul et al. 1999). Riboprobes labeled with $^{35}$S-UTP (uridine triphosphate) were prepared by *in vitro* transcription. Dot blots were analyzed using a BioRad Model GS363 Molecular Imager. Standard curves were made from opposite orientation *in vitro* transcripts generated from the same *rbcL* clones used to make the ribo-probes.

**Flow cytometry.** Samples of 1 ml were fixed with 20 µl of 10% para-formaldehyde and frozen in liquid nitrogen. *Prochlorococcus*, *Synechococcus* and picoeucaryotic algal populations were then quantified using a Becton Dickinson FACSCalibur flow cytometer outfitted with a 488 nm, 15 mW Argon laser. Forward angle light scatter, right angle light scatter, green (530 ± 30 nm), orange (585 ± 30 nm) and red (650 ± 30 nm) fluorescence parameters were collected for each event. Purple-yellow calibration beads (2.2 µm, Spherotech) were added to each sample to permit normalization of all fluorescence signals. Data were collected using CellQuest™ software (V. 3, Becton Dickinson 1996) and analyzed using CYTOWIN software (Vaulot et al. 1989); www.sb-roscoff.fr/Phyto/cyto.html #cytowin). Event rates were recorded for each sample and abundances were corrected for volume analyzed and enumeration efficiency factor. The efficiency factor was calculated from event rate and counts for series of known concentrations of calibration beads.

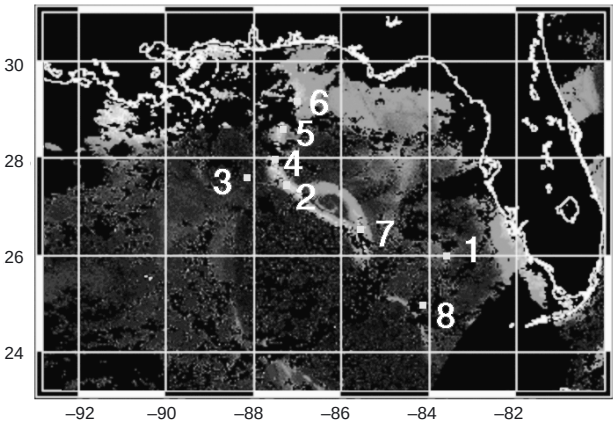


Fig. 1. Chlorophyll *a* surface concentrations as measured by SeaWiFS (Sea-Viewing Wide Field-of-view Sensor) in our sampling region. Image was converted to black and white. A color image showing the plume can be found in Wawrik & Paul (2004, this issue). Lighter shades of gray indicate higher surface chl *a*. Concentrations in near-shore regions are often heavily influenced and potentially biased by the presence of colored dissolved organic matter, bottom reflectance and suspended sediment. Land and clouds are black. Station locations are indicated

**Assessment of nutrient limitation.** To investigate potential nutrient limitation ratios of DIN, phosphate and silicate were calculated and compared to typical Redfield ratios. Ratios of N:P were determined by adding measurements of ammonium and nitrate + nitrite ($NH_4$ + NOx). Ratios of N:P ≥ 30 were taken to potentially indicate phosphate limitation, and ratios ≤10 to indicate potential nitrogen limitation (Healey & Hendzel 1979, Healey 1985, Suttle & Harrison 1988, Dortch & Whitledge 1992). Ratios of Si:N much greater than 1 are thought to indicate nitrogen limitation, whereas ratios much smaller than 1 may indicate silicate limitation (Brzezinski 1985, Levasseur & Therriault 1987, Dortch & Whitledge 1992). For the purpose of this study, Si:N ratios of 0.8 and 1.2 were assumed to indicate silicate and nitrogen limitation, respectively. Silicate limitation may also be indicated by Si:P ratios ≥3 (Harrison et al. 1977, Dortch & Whitledge 1992).

## RESULTS

Sampling was initiated on the West Florida Shelf and proceeded from offshore to onshore along the plume axis (Fig. 1). On our return trip, we sampled the most distal plume station (Stn 7) and an offshore/oligotrophic reference station (Stn 8). Stns 1 and 8 were outside the plume while Stns 2 to 7 were located within. A discussion of salinity, productivity and composition of phytoplankton along this transect together with a more detailed summary of our sampling strategy is found in the companion paper (Wawrik & Paul 2004).

### Nitrate and ammonium concentrations

Ammonium concentrations varied 2.5-fold and ranged between 0.18 and 0.44 µM (Fig. 2A). No discernable pattern in the variability of ammonium concentrations was observed. Nitrate concentrations varied much more dramatically and increased 69-fold from 0.036 to 2.5 µM between Stn 8 and their peak at Stn 4 (Fig. 2A). Nitrate concentrations were significantly greater in plume samples than in non-plume samples (Student's $t$ = 1.9, p = 0.1). Nitrate concentrations were significantly greater in samples from Stns 2 through 5 than in the remaining plume samples ($t$ = 5.9, p = 0.004).

### Ammonium and nitrate uptake

Despite greater nitrate concentrations in the plume, ammonium uptake was much higher than nitrate uptake. Ammonium uptake ranged between 16.5 nM $h^{-1}$

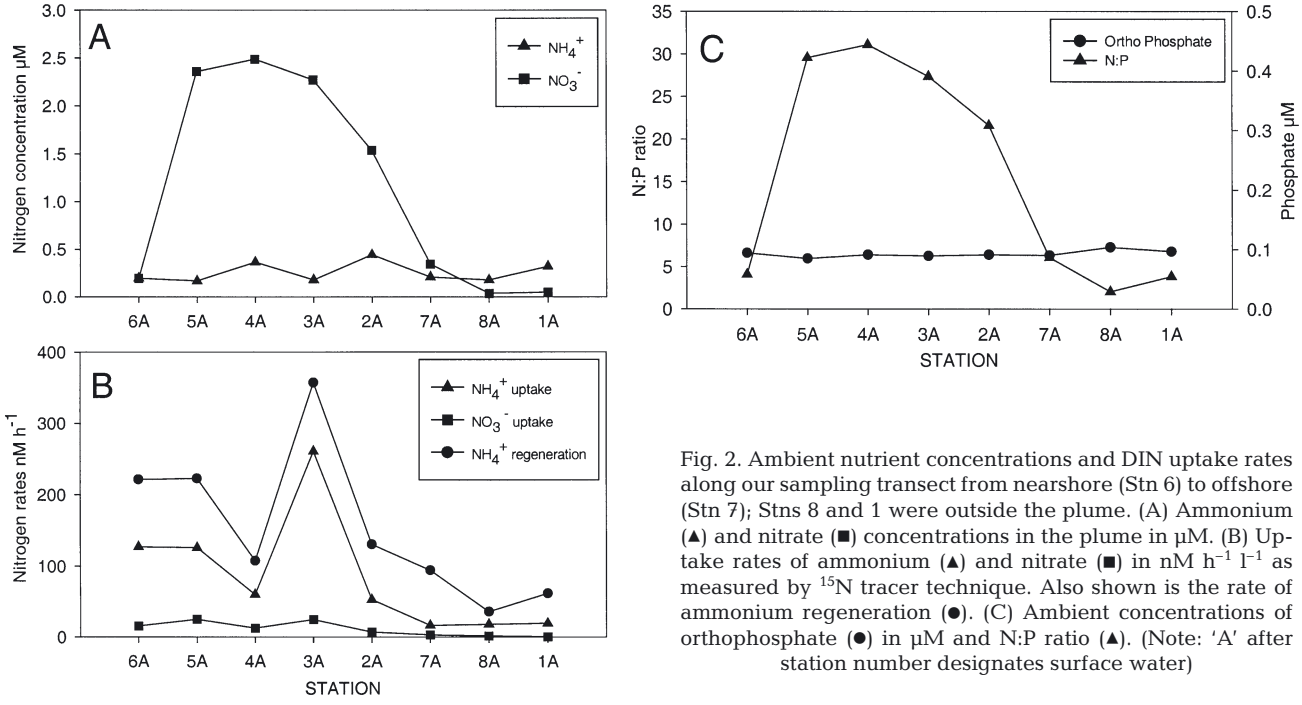


Fig. 2. Ambient nutrient concentrations and DIN uptake rates along our sampling transect from nearshore (Stn 6) to offshore (Stn 7); Stns 8 and 1 were outside the plume. (A) Ammonium (▲) and nitrate (■) concentrations in the plume in µM. (B) Uptake rates of ammonium (▲) and nitrate (■) in nM $h^{-1}$ $l^{-1}$ as measured by $^{15}N$ tracer technique. Also shown is the rate of ammonium regeneration (●). (C) Ambient concentrations of orthophosphate (●) in µM and N:P ratio (▲). (Note: 'A' after station number designates surface water)

at our most oceanic plume station (Stn 7) and 260 nM $h^{-1}$ at Stn 3 and increased toward the Mississippi Delta (Fig. 2B). Ammonium uptake in non-plume samples averaged 18.7 nM $h^{-1}$. Nitrate uptake rates ranged between 3.1 and 25 nM $h^{-1}$, and were on average almost 7-fold lower than ammonium uptake rates within the plume and almost 24-fold lower outside. Uptake averaged 1.03 nM $h^{-1}$ in non-plume samples. Data analysis also revealed that nitrate uptake was significantly correlated to the ratio of dissolved nitrate to dissolved ammonium ($R^2 = 0.79$, $p = 0.003$). The exception was Stn 6, where an intermediate nitrate uptake rate of 15.7 µM $h^{-1}$ coincided with the lowest nitrate concentration measured anywhere in the plume. Nitrate uptake rates were significantly correlated to ammonium uptake rates along our transect ($R^2 = 0.78$, $p = 0.004$).

Table 1. Nitrate (N), phosphate (P) and silicate (Si) limitation in the plume. N:P, Si:N and Si:P ratios are shown. Based on these ratios limitation by N, P and Si is determined using published ratios. +: limitation by a particular nutrient; –: no evidence for limitation observed based on nutrient ratios

| Stn | N:P | Si:N | Si:P | N:P | | Si:N | | Si:P |
|---|---|---|---|---|---|---|---|---|
| | | | | P | N | N | Si | Si |
| 6 | 4.09 | 0.87 | 3.55 | – | + | – | – | – |
| 5 | 29.58 | 0.36 | 10.60 | + | – | – | + | – |
| 4 | 31.11 | 0.06 | 1.84 | + | – | – | + | + |
| 3 | 27.36 | 0.56 | 15.46 | – | – | – | + | – |
| 2 | 21.59 | 0.33 | 7.16 | – | – | – | + | – |
| 7 | 6.07 | 0.42 | 2.54 | – | + | – | + | + |
| 8 | 2.04 | 5.00 | 10.22 | – | + | + | – | – |
| 1 | 3.83 | 1.34 | 5.12 | – | + | + | – | |

### Phosphate and silicate

Phosphate concentrations along the transect were between 0.085 and 0.104 µM (Fig. 2C). Concentrations were significantly higher in non-plume samples relative to plume samples ($t = 3.4$, $p = 0.01$). The N:P ratio was calculated by adding dissolved nitrate and ammonium concentrations and dividing this number by dissolved phosphate (Fig. 2C). Silicate varied more than 8-fold within the plume, ranging between 0.17 µM at Stn 4 and 1.38 µM at Stn 3. There was no obvious pattern of silicate concentrations along the plume, and concentrations at non-plume stations were not significantly different from those within the plume.

Analysis of nutrient ratios indicated that phosphate limitation of phytoplankton growth may have occurred at Stns 4 and 5 (Table 1). Based on N:P and Si:N ratios, nitrogen was limiting at non-plume Stns 1 and 8. Ratios of N:P also suggested potential nitrogen limitation at Stns 6 and 7. There was no evidence for nitrogen limitation at the remaining plume stations, which contained ample nitrate. Based on Si:N and Si:P ratios, silicate appeared limiting in the central portion of the plume surveyed here. Non-plume stations did not appear to be silica limited.

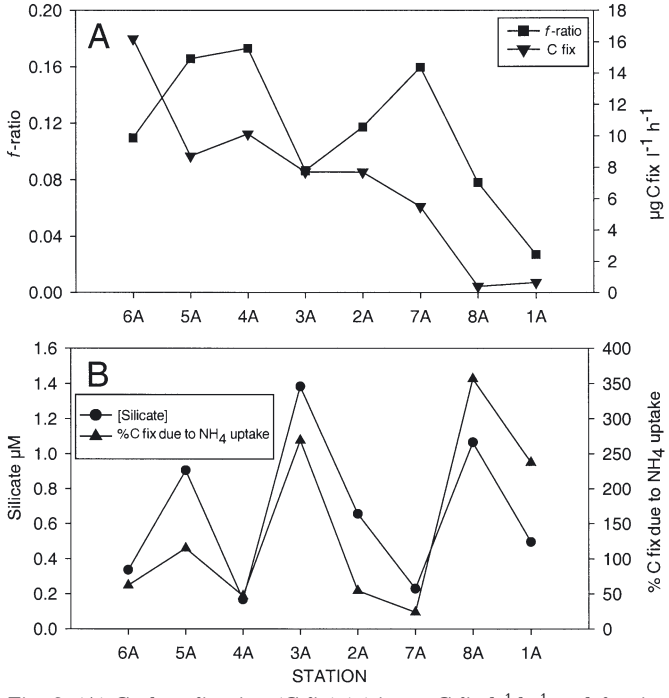


Fig. 3. (A) Carbon fixation (C fix) (▼) in µg C fix $l^{-1}$ $h^{-1}$ and *f*-ratio (■) along our transect. The *f*-ratio is calculated as the ratio between nitrate and nitrate + ammonium uptake. (B) Ambient silicate concentrations (●) in µM and the % C fix accounted for by ammonium uptake (▲) assuming a Redfield ratio of 6.6 for C:N uptake. (Note: 'A' after station number designates surface water)

### Carbon fixation and *f*-ratio

Carbon fixation in non-plume samples averaged 0.53 µg C $l^{-1}$ $h^{-1}$ and steadily increased almost 25-fold along the plume to a rate of 16.2 µg $l^{-1}$ $h^{-1}$ at Stn 6 (Fig. 3A). Variability in carbon fixation along the transect was not correlated with ammonium or nitrate uptake, but rather displayed a negative correlation with salinity ($R^2$ = 0.74, p = 0.006). The *f*-ratio is the fraction of 'new' to total production, and is calculated by dividing nitrate uptake by nitrate plus ammonia uptake (Eppley 1981). The *f*-ratios were lowest in non-plume samples, where they averaged 0.053, and were significantly higher in the plume ($t$ = 2.8, p = 0.029). There was no significant correlation of the *f*-ratio to any other parameter described here. Assuming that all ammonium uptake leads to primary production in the plume, and assuming a Redfield ratio of 6.6 for C:N uptake, we calculated the percent carbon fixation due to ammonium assimilation (Fig. 3B). This percentage was significantly correlated to ambient silicate concentrations in our sampling region (p = 0.034, $R^2$ = 0.75).

### Flow cytometry

*Synechococcus* was more abundant at the more coastal stations, reaching a maximum of $2.3 \times 10^5$ cells $ml^{-1}$ at Stn 5, but declining in the central portion of the plume (Fig. 4A). At Stns 2 and 7, *Synechococcus* was no longer significantly elevated over surface abundance at non-plume sites. With the exception of Stn 3, *Prochlorococcus* was not abundant in plume surface samples. Counts in the plume were $<10^4$ cells $ml^{-1}$ at Stns 2, 6 and 7 and $<2 \times 10^4$ cells $ml^{-1}$ at Stns 4 and 5. At Stn 3, *Prochlorococcus* was present at an abundance of $1.68 \times 10^5$ cells $ml^{-1}$. This cell density was even 2.6-fold greater than concentrations observed at the most oligotrophic site (Stn 8). The picoeukaryotes fraction counted by flow cytometry includes small red-fluorescing cells such as prymnesiophytes, pelagophytes, diatoms, cryptophytes, chlorophytes and others. The concentration of picoeukaryotes increased steadily from offshore to onshore ranging between $9.5 \times 10^2$ cells $ml^{-1}$ and $4.5 \times 10^3$ cells $ml^{-1}$. The abundance of picoeukaryotes was significantly positively correlated with rates of carbon fixation ($R^2$ = 0.86, p = 0.001) and negatively correlated with salinity ($R^2$ = 0.80, p = 0.003).

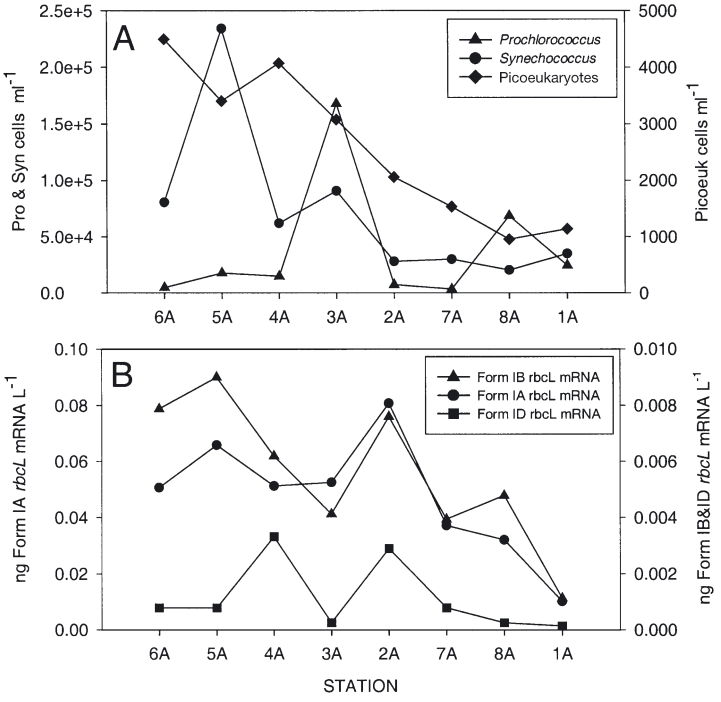


Fig. 4 (A) Results from flow cytometry analysis. Shown are *Prochlorococcus* (▲), *Synechococcus* (●) and the number of picoeukaryotes (◆) $ml^{-1}$. (B) Detected levels of form IA (●), form IB (▲) and form ID (■) *rbcL* mRNA along our sampling transect. (Note: 'A' after station number designates surface water)

### *rbcL* mRNA expression

The dominant *rbcL* transcript observed at all stations sampled was the alpha-cyanobacterial form IA of this gene. Form IA *rbcL* mRNA concentrations ranged between 0.01 ng $l^{-1}$ at Stn 1 and 0.081 ng $l^{-1}$ at Stn 2, and in average exceeded form IB and ID transcript levels by 8.8- and 8.0-fold, respectively. Form IA and IB *rbcL* mRNA concentrations were well correlated ($R^2$ = 0.84, p = 0.008) and increased significantly between the west Florida shelf and the Mississippi Delta (Fig. 4B). Form IB expression, which ranged between 0.0011 ng $l^{-1}$ at Stn 1 and 0.0090 ng $l^{-1}$ at Stn 5, was also significantly correlated to carbon fixation ($R^2$ = 0.70, p = 0.05), while form IA and form ID were not. The greatest range in expression values was observed for form ID transcript, which varied almost 24-fold between lowest values at Stn 1 (0.0014 ng $l^{-1}$) and their high at Stn 4 (0.033 ng $l^{-1}$). Form ID expression was significantly correlated to *in situ* concentrations of ammonium ($R^2$ = 0.74, p = 0.037) and the ratio of nitrate to nitrate uptake ($R^2$ = 0.84, p = 0.008). We observed no significant correlations between the forms of *rbcL* quantified here and our flow cytometry counts for picoeukaryotes, *Synechococcus* and *Prochlorococcus*.

## DISCUSSION

The Mississippi River plume, extending westward on the continental shelf, has been studied intensely. Data presented here represents the first targeted survey and transect of the relatively high-salinity and offshore portion of the Mississippi plume that periodically wanders into the oligotrophic NE GOM.

Low ammonium concentrations have been reported for the plume (0.29 to 2.5 µM) along the entire salinity gradient (Pakulski et al. 1995). In transects extending offshore from the Southwest Pass and the Atchafalaya River, ammonium concentrations ranging between 0 and 2.6 µM were measured, with concentrations peaking at mid salinities (Gardner et al. 1997). Similar results (with concentrations up to 3.58 µM ammonium at mid salinities) were obtained in a similar survey of the Southwest Pass discharge region (Bode & Dortch 1996). Ammonium concentrations reported here for the offshore plume were in the lower range of these previously reported values. Potential ammonium uptake rates in the coastal Mississippi River plume have been reported in the range between 0.4 and 1.8 µM $h^{-1}$ (Gardner et al. 1997) and up to 4.4 µM $h^{-1}$ (Bode & Dortch 1996), while ammonium regeneration rates ranged between 0.08 and 0.75 µM $h^{-1}$ (Gardner et al. 1997) and 0.03 and 1.09 µM $h^{-1}$ (Bode & Dortch 1996). Both studies observed peak uptake and regeneration rates at intermediate salinities. Ammonium uptake and regeneration rates reported here are consistent with these observations recorded previously and support the hypothesis that the most intense recycled production occurs at intermediate salinities. In the offshore plume, nitrate exceeded ammonium at all but our most inshore plume station, while ammonium exceeded nitrate concentrations at our non-plume oligotrophic stations. Despite higher nitrate concentrations, submicromolar levels of ammonium were the preferred source of nitrogen, and production was fueled by high levels of ammonium regeneration (Fig. 1C). The dominance of regenerated production in the offshore plume is in contrast to the coastal plume, which exhibits high rates of nitrate driven new production.

Not all nitrate uptake represents new production, however. Evidence has been reported for intense nitrification in the plume, particularly at intermediate salinities (Pakulski et al. 1995). Nitrate found in offshore plume waters may thus be, at least in part, recycled, blurring the distinction between new and recycled production commonly used. It should be noted that, if this were the case, our nitrate uptake measurements may be an underestimate of actual rates, due to isotope dilution of the nitrate pool during incubations. One possible indication for nitrification is the strong non-conservative mixing behavior of nitrate concentrations along our sampling transect (with peak values at Stns 4 and 6). It is possible that this increase in nitrate was due to the activity of nitrifying bacteria, although Mississippi outflow heterogeneity should not be discounted.

Ammonium is thought to be the preferred source of nitrogen for phytoplankton growth. This preference is mediated by specific cell-surface associated transporters, which follow substrate dependent Michaelis-Menten enzyme kinetics. It is further thought that oceanic species are adapted to their environment by possessing high substrate affinities (Dugdale & Goering 1967) and that nitrate uptake may be dramatically reduced by the presence of even low concentrations of ammonium (Wheeler & Kokkinakis 1990). In the field, uptake kinetics of natural phytoplankton assemblages have been studied by the addition of 10 to 1000 nM $^{15}NH_4^+$ and $^{15}NO_3^-$ (Harrsion et al. 1996). Almost without exception, the Michaelis-Menten equation was an appropriate descriptor of uptake kinetics in samples from a wide range of physical, chemical and biological conditions. Ammonium was preferred over nitrate across a large spectrum of nitrogen concentrations and inhibited nitrate uptake with an inhibition half-saturation parameter ($K_i$) of 40 to 50 nM. Significant inhibition of nitrate uptake by ammonium has also been reported by other authors (Wheeler & Kokkinakis 1990). Ammonium only accounted for <1% of total

DIN, yet accounted for 44 to 89% of total N assimilation, and nitrate assimilation was negatively correlated with ambient ammonium concentrations (Wheeler & Kokkinakis 1990). These observations held only partially true for the offshore Mississippi River plume stations of this study where ammonium uptake was clearly dominant, despite low concentrations. Nitrate uptake in the plume, however, was both positively and significantly correlated with ammonium uptake and the nitrate to ammonium ratio. One possible explanation for this observation may be that nitrate transport and reduction pathways are expressed only when sufficient nitrate is present and when ammonium concentrations are insufficient to repress their expression. As a result, a high ratio of nitrate to ammonium combined with more favorable uptake enzyme kinetics and cellular demand could result in conditions more favorable to the utilization of nitrate.

Nutrient discharge by the Mississippi River has been implicated in sustaining high levels of primary productivity in the northern GOM (Riley 1937, Sklar & Turner 1981, Lohrenz et al. 1990, Redalje et al. 1994, Wawrik et al. 2003). As Mississippi River water enters the northern GOM, it carries a high load of nitrate (between 20 and 200 µM, depending on the season; Lohrenz et al. 1999), but only low concentrations of orthophosphate (between 0.3 and 5 µM; Lohrenz et al. 1999). Ammonium as well as nitrite are also not present in significant quantities (Antweiler et al. 1995). Productivity in the discharge area is initially limited by the availability of light due to high turbidity of the river water. As a result of the interplay between the availability of nutrients and turbidity, the highest productivity in the plume is most often found in regions of intermediate salinities to be between 10 and 30‰ (Lohrenz et al. 1990, 1999, Dagg & Whitledge 1991, Dortch & Whitledge 1992, Hitchcock & Whitledge 1992), where high nutrient water is no longer limited by light availability. Additionally, factors other than light have also been implicated in constraining biomass and productivity, even in the most turbid portions of the plume (Lohrenz et al. 1990). Both phosphate and silicate have been found to limit phytoplankton productivity in the Mississippi Delta region (Smith & Hitchcock 1994, Nelson & Dortch 1996). As river water mixes into the oceanic end-member, nitrate and silicate are rapidly depleted from the plume, while supporting intense new production. Nitrate concentrations usually approach the limits of detection at salinities greater than 30 to 33‰ (Lohrenz et al. 1990).

It has been hypothesized that at least 1 or more nutrients (in particular nitrogen) will eventually become limiting in the plume (Sklar & Turner 1981, Lohrenz et al. 1990), as has been observed for silicate in the Hudson River plume (Malone et al. 1980). Dortch & Whitledge (1992) specifically addressed the hypothesis that nitrogen or silica may become limiting to productivity in the Mississippi River plume and nearby regions. Using ratios of cellular-free amino acids to protein (AA/Pr) as well as ambient nutrient concentrations, they concluded that nitrogen limitation was not wide spread in the plume and was most likely to occur during summer months at high salinities. Nutrient ratios indicated that silicate was at least as likely to be limiting.

Although nutrient ratios should be interpreted with caution, since they only possess limited use as a predictor of nutrient limitation, several observations can be made based on our measurements (Table 1). Nitrogen appeared not to be limiting phytoplankton biomass in the plume, at least at stations with ≥1 µM nitrate. This notion is supported by ammonium regeneration rates, which were on average 2.5-fold greater than ammonium uptake in plume samples, indicating that the rate of DIN supply was more than sufficient to support the observed rates of primary production. The nutrient most likely limiting in the offshore plume, based on nutrient ratios, was silicate, followed by or in combination with phosphate. Phosphate was relatively depleted and near the detection limits at all our stations, indicating that it may have been a limiting nutrient throughout. N:P ratios however indicated that phosphate may have been particularly scarce at stations where *Synechococcus* was dominating over diatoms (Stns 3 through 5; Wawrik & Paul 2004). During the past century, nitrate loading of the Mississippi River has at least doubled, while silica concentrations have been reduced by half, reducing the Si:N ratio in river discharge from 4:1 to 1:1 (Turner & Rabalais 1991, Rabalais et al. 2002). These changes potentially influenced the size and type of diatoms found in the Mississippi watershed, favoring small, less heavily silicified forms or even non-silicified phytoplankton. Silica limitation, however, is not thought to ultimately limit phytoplankton biomass and may only result in the adjustment of the ambient species composition, which could have large implications for food web dynamics, nutrient cycling and the rate of carbon sequestration. Also, less silicified populations of diatoms may exhibit lower cellular Si:N ratios, potentially alleviating silicate limitation in the plume.

Analysis of pigment data and the composition of *rbcL* cDNA clone libraries obtained from our plume samples, indicated that the offshore plume was divided into a more coastal diatom dominated, a central *Synechococcus* dominated and a more oceanic diatom dominated region (Wawrik & Paul 2004). Flow cytometry supports these observations (Fig. 4A). Numerically, *Synechococcus* was the principle phytoplankter at all plume stations and was particularly

abundant at intermediate salinity stations (30.8 to 31.5‰), where pigments indicated their dominance (Wawrik & Paul 2004). Similar observations for *Synechococcus* have been reported for the dilution zone of the Yangtze River, China, where abundance ranged between $10^2$ and $10^5$ cells $ml^{-1}$ in the summer (Vaulot & Xiuren 1988) and increased in the offshore direction. Highest abundance was observed at salinities between 25 and 30‰. Further offshore plume stations were dominated by diatoms as indicated by pigment ratios (Wawrik & Paul 2004), despite indications of silica limitation in this region. This suggests that factors other than silica or nitrogen limitation may be controlling phytoplankton composition and dynamics in the most offshore portion of the plume. This point is particularly well illustrated by the observation of a large population of *Prochlorococcus* at Stn 3, which coincided with the highest ammonium uptake rates measured in the plume. Stn 3, which was located on the edge of the plume, contained a phytoplankton community most similar to our non-plume stations (Wawrik & Paul 2004), but otherwise exhibited characteristics (salinity, nutrient concentrations, ammonium uptake rate and productivity) very similar to adjacent and more centrally located Stns 2 and 4. The large numbers of *Prochlorococcus* at Stn 3 is a somewhat surprising finding, since this organism is typically not abundant in plume surface waters (Wawrik et al. 2003).

It is also interesting to note that despite the numerical dominance of *Synechococcus* in the plume, carbon fixation was most tightly correlated with flow cytometry counts for picoeukaryotes. Unfortunately we performed no size-fractionation experiments and it was thus difficult to assign either productivity or nitrogen uptake measurements to individual components of the phytoplankton using our data. Clone library data indicated the presence of a large number of diatom species, which may have dominated some portions of the plume based on pigment information (Wawrik & Paul 2004). Since diatoms are thought to be capable of supporting their growth using ammonium, it is possible that the high rates of ammonium uptake and primary productivity we observed were at least in part due to the presence of a diverse group of these organisms. *Phaeodactylum tricornutum*, for example, has been shown to assimilate both L-arginine and ammonium simultaneously and individually at a rate sufficient for growth (Flynn & Wright 1986). Alternatively, a diverse group of green algae was also present in the plume and was actively transcribing their carbon fixation genes. Form IB *rbcL* mRNA concentrations were significantly correlated with carbon fixation ($R^2 = 0.5$, $p = 0.05$, $N = 8$), while form ID (diatom/chromophytes) *rbcL* was not.

The most abundant form of *rbcL* transcript found at all stations was nonetheless the form IA (α-cyanobacterial) type, corroborating the observation that *Synechococcus* numerically dominated that plume. Both the cyanobacterial and chromophytic algal *rbcL* forms (form IA and ID respectively) were not significantly correlated with carbon fixation, exemplifying the highly variable abundance and contribution to total chl *a* of these organisms in our sampling region. In addition, it is possible that chemolithotrophic nitrifying bacteria (which also contain a form IA or the RubisCO gene) may have influenced our form IA signal and obscured its correlation with productivity. Considering evidence of nitrification in the plume (Pakulski et al. 1995), it is feasible that high levels of form IA *rbcL* were in part due to the presence of such organisms. Form ID *rbcL* expression was the most variable, increasing 24-fold between non-plume and plume stations. Expression was significantly greater in the plume, but most tightly correlated to the ratio between dissolved nitrate and nitrate uptake, indicating that chromophytic algae were particularly successful where other nitrate using phytoplankton may have been less abundant or less active.

Together, these observations suggest that an intense bloom of diatoms in the near-shore plume (based upon coastal sampling by others), where nitrate levels are high and silica is not limiting, is replaced by smaller, ammonium preferring cells, in particular *Synechococcus* as plume water moves into the oligotrophic GOM. New production becomes negligible as recycling of sub-micromolar ammonium ensues in the offshore portion of the plume.

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