

Mineralization of Organic Material and Bacterial Dynamics in Mississippi River Plume Water

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ABSTRACT: Net remineralization rates of organic matter and bacterial growth rates were observed in dark-bottle incubation experiments conducted in July–August and February with water samples collected from sites in the Mississippi River plume of the Gulf of Mexico. Our objectives were to measure site-specific degradation rates of labile dissolved and particulate organic matter, quantify the potential importance of bacteria in these processes, and examine the kinetics of degradation over time. Unfiltered samples, and samples treated to remove (or dilute out) particles larger than bacteria, were enclosed in 9-l bottles and incubated in the dark for 3–5 d. Respiration rates and inorganic compound accumulation rates were higher in summer than in winter and were highest in unfiltered surface samples at sites of intermediate salinities where phytoplankton were most abundant. The ratio of ammonium accumulation to oxygen removal in summer experiments suggested that the mineralized organic material resembled “Redfield” stoichiometry. Chemical fluxes were greater in bottles containing large (>1–3 μm) particles than in the bottles with these particles removed, but bacterial activities were generally similar in both treatments. These results suggest that particle consumers were an important component of total organic matter degradation. However, these experiments may have underestimated natural bacterial degradation rates because the absence of light could affect the production of labile organic substrates by phytoplankton. In agreement, with this hypothesis, bacterial growth rates tended to decrease over time in summer in surface plume waters where phytoplankton were abundant. In conjunction with other data, our results indicate that heterotrophic processes in the water column are spatially and temporally dependent on phytoplankton production.

Introduction

Inputs of nitrogen and other nutrients from the Mississippi River greatly enhance algal productivity in the river plume relative to other regions in the Gulf of Mexico (Lohrenz et al. 1990, 1992). Heterotrophic processes such as bacterial activity (Chin-Leo and Benner 1992) and community nutrient regeneration rates (Cotner and Gardner 1993) are also elevated in the mid-salinity regions of the plume. The river supplies inorganic and organic nutrients that can be used by phytoplankton (Riley 1937; Sklar and Turner 1981) and bacteria

(Findlay et al. 1992). In addition, organic materials produced by phytoplankton in the plume can be used by bacteria (Chin-Leo and Benner 1992) and zooplankton (Dagg et al. 1987; Dagg and Ortner 1992; Fahnenstiel et al. 1992). An understanding of the dynamics of nutrient cycling and organic matter degradation in different regions of the plume is needed to evaluate sources of nutrients to phytoplankton and to determine mechanisms of oxygen depletion from the water. Seasonal hypoxia is a recurring problem affecting benthic biota and fish populations (Harper et al. 1981; Pavela et al. 1983) in some relatively shallow regions of the plume downstream from Mississippi River outflows (Rabalais et al. 1991, 1992).

Information about autotrophic-heterotrophic interactions can be obtained by observing the turn-

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TABLE 1. Source description, salinity, total incubation time, and initial dissolved organic carbon (DOC) concentration of the waters used in dark-bottle incubations conducted in July–August 1990 and February 1991. All waters were from near-surface except for the bottom water collected in the hypoxic region. See Fig. 1 for sampling site location.

Description	Salinity (‰)	Incubation Time (h)	DOC Concentration (mg C l ⁻¹)
Summer 1990			
River	0.2	88	4.14
Plume 1	16.7	90	3.43
Plume 2	17.4	99	3.34
Hypoxic region			
Surface	23.1	117	2.98
Bottom	35.7	93	1.22
Slope	36.1	72	0.79
Winter 1991			
River	0.3	72	3.22
Plume 1	17.0	63	2.02
Plume 2 (Hypox. region)	28.3	48	1.64
Slope	34.0	48	1.06

over rates of organic material in water from different regions of the plume. Under in situ conditions, organic matter production by autotrophs and degradation by heterotrophs occur simultaneously, but these processes must be experimentally differentiated to determine rates of organic matter turnover (Cole et al. 1982; Kirchman et al. 1991). These processes can be differentiated by monitoring changes in concentrations of oxygen, inorganic mineralization products (e.g., CO₂ and NH₄⁺), and bacterial abundances and growth rates in bottles held in the dark to stop autotrophic production of organic material during incubations (Von Brand et al. 1937, 1942; Grill and Richards 1964; Gardner et al. 1987, 1989). This approach provides information on the reactivity, elemental composition, sources, and fate of labile organic matter in the samples. Kinetic changes in rates during the course of a dark incubation can also suggest potential relationships between organic matter degradation and photosynthesis.

In this paper, we examine organic matter degradation in the river outflow and in different regions of the plume by following mineralization and bacterial production rates as well as oxygen consumption rate in dark bottles during incubations of 3–5 d. Specific questions addressed are 1) What are the relative rates of organic matter degradation in the Mississippi River, the plume, and offshore waters in summer and winter? 2) What percentage of total organic matter degradation can be accounted for by bacterial processes? and 3) What are the kinetics of organic matter degrada-

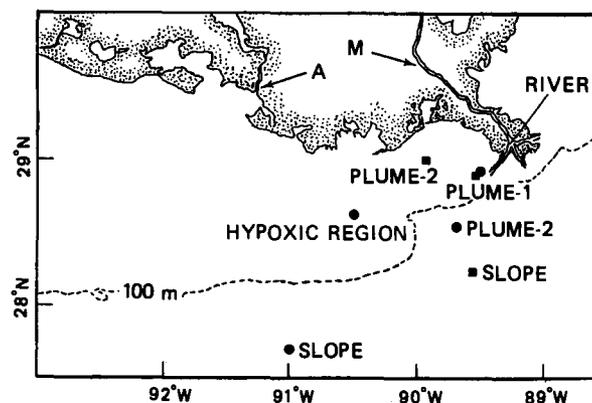


Fig. 1. Map of the area surveyed on the Louisiana Shelf showing the location of the Mississippi River (M), the Atchafalaya River (A), and the location of stations where water samples were collected for the mesocosm experiments. Filled circles represent stations sampled during July and August 1990 and filled squares represent stations sampled in February 1991. The river samples were collected at the Head of Passes (RIVER) in the Mississippi Delta.

tion with time after removal of the samples from light?

Materials and Methods

SITE DESCRIPTION AND SAMPLING PROCEDURES

Experiments were conducted in summer (July 18–August 8, 1990) and winter (February 19–28, 1991) on the National Oceanic and Atmospheric Administration (NOAA) vessel *Malcolm Baldrige*. Water samples were collected from selected sites of increasing salinities (0–36‰; Table 1) in an area of the northern Gulf of Mexico extending offshore from the Southwest Pass of the main delta of the Mississippi River (Fig. 1).

EXPERIMENTAL MICROCOSMS

Microcosm experiments were conducted in 9-l glass bottles (Fig. 2) that were closed to the outside

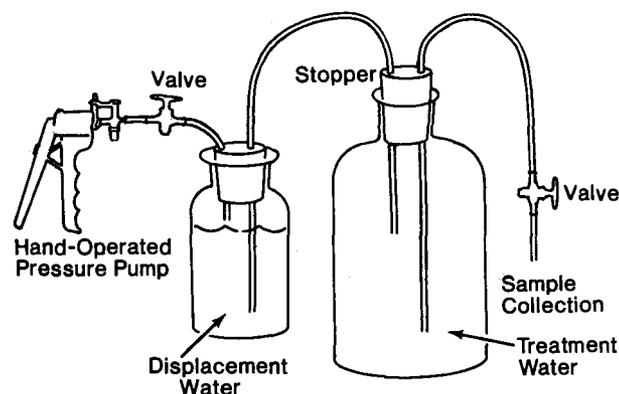


Fig. 2. Schematic diagram of dark-bottle microcosms.

atmosphere and incubated in the dark for 3–5 d at in situ temperatures. For each experiment, duplicate bottles were set up for both filtered and “whole” (i.e., unfiltered) water samples. The filtered samples were prepared either by gently pumping the seawater through a Nuclepore cartridge filter (1- μm pore-size; summer cruise) or by diluting (1:5) 3- μm pore-size filtered water with 0.2- μm pore-size filtered seawater from the same site (winter cruise). Both approaches decreased the quantitative importance of large constituents (i.e., zooplankton, large phytoplankton, and detrital particles) relative to bacterial-sized particles. A 2-l reservoir, attached to each experimental bottle, provided displacement water (without leaving an air space) for samples removed from the bottles. The total water volume displaced before the last sampling point was about 1 l.

Each bottle was sampled 1 or 2 times per day over the 3-d to 5-d incubation periods. Dissolved organic carbon (DOC) concentrations were measured in the initial treatment waters, and concentrations of ammonium, dissolved oxygen, and carbon dioxide were monitored over time for each treatment to estimate net mineralization rates for organic carbon and nitrogen in samples from the various sampling sites. Site comparisons were made using accumulation or removal rates obtained over the entire incubation periods.

CHEMICAL AND BACTERIAL ANALYSES

Ammonium concentrations were measured fluorometrically after separation from amino acids by high performance cation exchange chromatography and reaction with o-phthalaldehyde/2-mercaptoethanol reagent (Gardner 1978; Gardner and St. John 1991). Dissolved organic carbon concentrations were measured using a Shimadzu TOC 5000 analyzer with a Pt catalyst (0.5% Pt on alumina) at 680°C (Benner and Strom 1993). Samples were filtered through a muffled (475°C) Whatman GF/F filter, acidified, and purged with ultra-high purity oxygen immediately prior to analysis. Standards were run several times each day, and the instrument and water blanks were evaluated during each cruise as described by Benner and Strom (1993). Dissolved oxygen was determined using the Winkler method as modified by Carrit and Carpenter (1966). Samples were collected in 300-ml BOD bottles and fixed with 1 ml of MnCl_2 (3 M) and 1 ml of NaOH-NaI (8 M–4 M) solution. After the samples were mixed and the precipitate allowed to settle, 1 ml of H_2SO_4 (10 N) was added and the sample was analyzed for dissolved oxygen. Fixed samples were titrated with an automated titration system controlled by an HP-85 computer (Friederich et al. 1984). Titrant ($\text{Na}_2\text{S}_2\text{O}_5$) was added by

a computer-controlled automated buret, and the end-point was detected colorimetrically using a light source and a photodiode sensor. Rates determined by this method agreed within 10% of replicate samples analyzed independently using a manually operated Winkler system with an optical end-point determination (Chin-Leo and Benner 1992). The respiration rate was determined by the change in oxygen concentration during the course of the experiments, assuming that rates were linear over time. Samples for dissolved inorganic carbon (DIC) were collected in clean glass scintillation vials (ca. 20 ml), poisoned with 20 μl of a saturated solution of HgCl_2 , sealed with a cone cap to eliminate any air, and refrigerated until analysis. In the laboratory, the sample was taken up in a 25-ml syringe and forced through a 12-ml loop attached to a modified high performance liquid chromatographic sampling valve. The sample was introduced into a stripping chamber, modified from Kroopnick (1974), containing 5 ml of 25% H_3PO_4 . The DIC was stripped through a water trap into a liquid nitrogen-cooled CO_2 trap using He. The frozen CO_2 was transferred to a cold finger and quantified using a Baratron Capacitance Manometer calibrated with known volumes of CO_2 . Precision (CV = 0.15%) was estimated by analyzing two sets (four samples each) of replicates from 300-ml BOD bottles. Duplicate measurements were run for all samples, and mineralization rates were based on the mean values.

Bacterial abundances were determined on DAPI-stained samples with epifluorescence microscopy (Porter and Feig 1980). Duplicate samples were analyzed, and bacteria were counted in at least 10 fields for each microscopic slide. Bacterial production rates were estimated from rates of DNA and protein synthesis as measured by uptake of saturating levels of [^3H]Thymidine (TdR) and [^{14}C]Leucine (Leu) (Chin-Leo and Kirchman 1988; Chin-Leo and Benner 1992). Water samples (10 ml) were incubated in triplicate with 10 nM (final concentration) ^3H -TdR (specific activity of 84.1 Ci mmol^{-1}), and 20 nM (final concentration) ^{14}C -Leu (specific activity of 328.5 mCi mmol^{-1}) for 30 min. All radioactive substrates were obtained from New England Nuclear (Boston, Massachusetts). The nucleic acid and protein fractions were separated, using a hot TCA extraction, to test for possible nonspecific incorporation of TdR into protein (Chin-Leo and Benner 1992). Replicate measurements of bacterial abundance and bacterial production (TdR and Leu incorporation) usually differed by <10% and <5%, respectively. All samples were corrected for abiotic adsorption by subtracting the radioactivity in formalin-killed controls from the uptake in live samples. Rates of bacterial

TABLE 2. Summary of rates of bacterial production, community respiration, and estimated minimum bacterial growth efficiencies during experiments conducted on the June–July 1990 NECOP cruise. Estimates of bacterial C production were derived from rates of TdR or Leu incorporation using conversion factors described in the text. Water samples were unfiltered (whole) or prefiltered through a 1- μm pore-size filter (filtered). Each number represents the mean from duplicate bottles. NA = not available.

Salinity (‰)	Temperature (°C)	Treatment	Bact. Prod'n ($\mu\text{g atom C l}^{-1} \text{ h}^{-1}$)	Respiration ($\mu\text{M O}_2 \text{ h}^{-1}$)	Bact. Growth Efficiency (%) TdR (Leu)
0.2	28	Whole	0.038	NA	NA
		Filtered	0.034	NA	NA
16.7	28	Whole	0.112	1.3	8 (9)
		Filtered	0.105	0.74	13 ^a (9) ^a
17.4	30	Whole	0.062	0.35	15 (22)
		Filtered	0.067	0.19	26 ^a (23) ^a
23.1	31	Whole	0.102	0.74	12 (18)
		Filtered	0.027	0.24	10 ^a (26) ^a
35.7	24	Whole	0.033	0.06	35 (66)
		Filtered	0.038	0.01	79 (92)

^a Values presented in Chin-Leo and Benner (1992).

C production were calculated using rates of TdR incorporation and an empirically-determined conversion factor of 1.89×10^{18} cells mol^{-1} and a biomass value of 20 fg C cell⁻¹ (Chin-Leo and Benner 1992). Independent estimates of bacterial C production were calculated using rates of Leu incorporation and the conversion factor of 3.1 kg C mol^{-1} determined by Simon and Azam (1989). A previous comparison showed an excellent correspondence between these independent measurements of bacterial C production in the Mississippi River plume (Chin-Leo and Benner 1992).

Estimates of bacterial production, used for site comparisons (Tables 2 and 3), were integrated over time. The values used for these integrations were the average of the duplicate bottles that were each sampled in triplicate. Estimates of bacterial growth efficiency or carbon conversion efficiency were conservatively calculated as:

$$\text{Growth efficiency} = \frac{\text{bacterial production}}{\text{bacterial production} + \text{oxygen consumption}}$$

assuming that the average respiratory quotient (RQ) was 1.0. This calculation is conservative because community respiration measurements would often overestimate bacterial respiration, particularly in unfiltered samples where the metazoans could represent an important component of total respiration. Also, the respiratory quotient would be less than 1.0 in situations where nitrification consumes significant oxygen.

Amino-acid turnover rates were measured in winter whole seawater samples by adding tracer amounts (less than 1 nM final concentration) of a

TABLE 3. Summary of rates of bacterial production, community respiration, carbon mineralization, and estimated minimum bacterial growth efficiencies during experiments conducted on the February 1991 NECOP cruise. Estimates of bacterial C production were derived from rates of TdR incorporation using conversion factors described in the text. Water samples were unfiltered (whole) or diluted (1:5) with 0.2- μm pore-size filtered water (filtered) from the same site. Each number represents the mean from duplicate bottles.

Sal. (‰)	Temp. (°C)	Treatment	Bact. Prod'n. ($\mu\text{g atom C l}^{-1} \text{ h}^{-1}$)	Respiration ($\mu\text{M O}_2 \text{ h}^{-1}$)	Mineralization ($\mu\text{g atom C l}^{-1} \text{ h}^{-1}$)	Bact. Growth Efficiency (%) TdR (Leu)
0.2	9	Whole	0.058	0.19	0.11	24 (40)
		Filtered	0.038	0.14	0.12	22 ^a (42) ^a
17.3	16	Whole	0.065	0.56	0.61	10 (15)
		Filtered	0.077	0.27	0.34	22 ^a (29) ^a
28.3	17	Whole	0.059	0.33	0.43	15 (23)
		Filtered	0.063	0.23	0.19	22 ^a (33) ^a
34.0	20	Whole	0.047	0.10	0.23	32 (37)
		Filtered	0.024	0.09	0.09	21 ^a (24) ^a

^a Values presented in Chin-Leo and Benner (1992).

mixture of ³H-labeled amino acids from algal protein hydrolysate (Amersham) to seawater and filtering 5 ml of sample through a 0.2- μm pore-size Millipore GS filter (Cotner and Gardner 1993). After radioisotope addition, samples were filtered at various intervals from 0 min to 30 min and dried. Biocount scintillation cocktail (RPI) was added, and samples were analyzed for radioisotopic uptake on a LKB Model 1217 Rackbeta liquid scintillation counter. Counts were corrected to disintegrations per minute (DPM) by calibration with quench curves and an external standard. Rates were calculated from points taken in the linear range of uptake.

Results

SITE-SPECIFIC RATES OF ORGANIC MATTER DEGRADATION

The water sampled over the salinity gradient had total DOC concentrations ranging from 3–4 mg C l^{-1} in the river to less than 1 mg C l^{-1} in an offshore station (Table 1). Decreases in oxygen concentrations and increases in other inorganic constituents in the filtered and unfiltered samples over the total incubation intervals provided information on the relative amounts and reactivity of labile organic materials that were present in the dissolved and particulate fractions from the different sites. Rates of oxidation were usually higher in bottles with unfiltered water than in bottles containing filtered water (Tables 2 and 3), indicating that detrital particles were important substrates for oxidation and/or that attached microflora and larger organisms were responsible for a significant fraction of the total respiration. Respiration rates ranged from 0.01 $\mu\text{M O}_2 \text{ h}^{-1}$ to 1.30 $\mu\text{M O}_2 \text{ h}^{-1}$

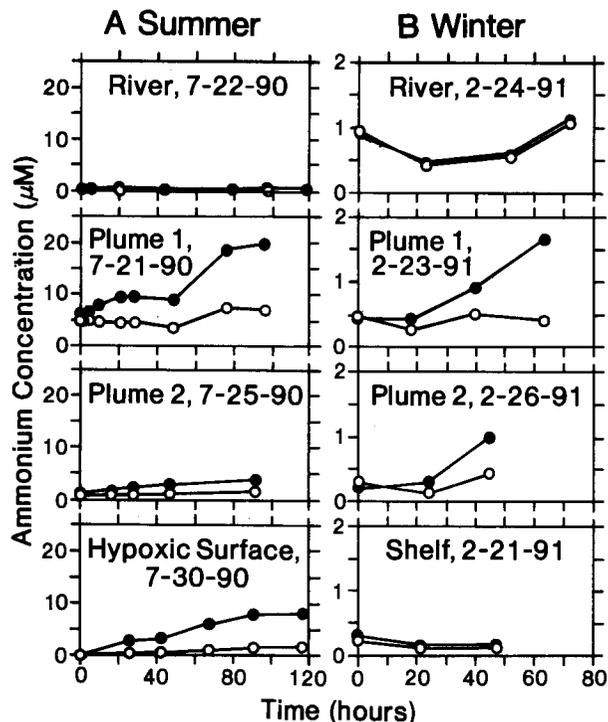


Fig. 3. Plots of ammonium accumulation over time in dark-bottle experiments. A.) Summer cruise. B.) Winter cruise. Closed circles represent data from unfiltered treatments. Open circles represent data from filtered treatments. Data from one of the summer filtered-river treatments was rejected and is not shown because ammonium accumulation and bacterial growth rates were higher than observed in either its duplicate treatment or in the whole-sample treatments from the same site. Note the order of magnitude difference in y-scales between winter and summer.

and were highest in unfiltered plume waters of intermediate salinities (17–28‰) during both the summer (Table 2) and winter (Table 3). During the winter cruise, both O_2 consumption and CO_2 production were measured as independent indicators of organic matter oxidation. Rates of O_2 consumption and CO_2 production were comparable, and the overall mean respiratory quotient (dCO_2/dO_2) was 0.99 ± 0.10 (SE, $n = 7$) if the value for the unfiltered bottle at 34‰ salinity is excluded (Table 3).

Duplicate-bottle ammonium regeneration rate results were averaged to compare treatment differences (Fig. 3) and to examine ammonium regeneration trends with salinity in summer and winter (Fig. 4). Ammonium concentrations either increased during the course of the incubations or showed little change over time (Fig. 3). In some offshore and bottom-water treatments, no ammonium was detected in the bottles either at the beginning or during the course of the experiments (data not shown in Fig. 3). In summer experi-

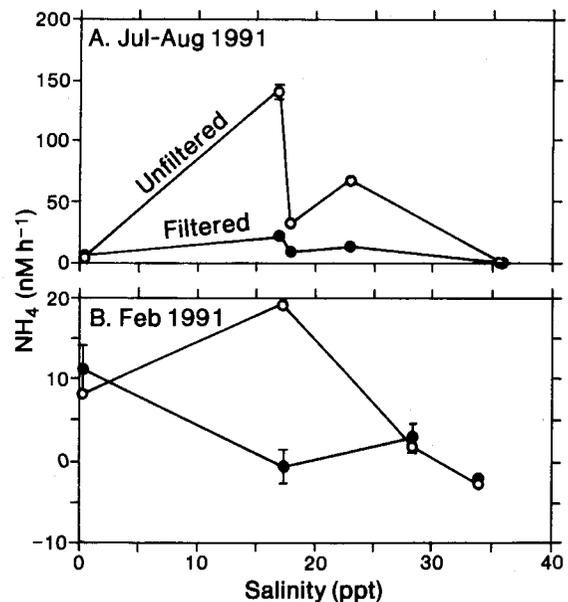


Fig. 4. Plots of mean net ammonium accumulation rates over time for dark-bottle experiments in summer (A) and winter (B). Note difference in y-scales.

ments, increases, when observed, were usually quite linear over time ($r > 0.90$) for the duration of the incubations. However, one of the plume samples (salinity 16.7‰) showed an apparent lag in ammonium accumulation during the second day (Fig. 3). In winter, ammonium accumulation rates were much lower than in summer, and accumulation showed a lag during the first 24 h in three out of four experiments.

In the summer, ammonium accumulation rates ranged from $<10 \text{ nM h}^{-1}$ for the river and offshore samples to about $30\text{--}150 \text{ nM h}^{-1}$ at the mid-salinity (17–23‰) plume stations (Fig. 4a). Ammonium accumulation rates in the winter ranged from slightly negative values at the offshore station to near 20 nM h^{-1} for the unfiltered water at the mid-salinity plume station (Fig. 4b). Winter experiments often did not show linear ammonium accumulation during incubations. The peak winter rate of ammonium accumulation in the mid-salinity plume was an order of magnitude lower than peak rates observed in the summer plume (Fig. 4). As was observed in the summer, differences between the unfiltered and filtered treatments were small except for the mid-salinity plume stations that accumulated ammonium in the unfiltered samples but showed little changes in the filtered treatments.

Comparison of ammonium accumulation rates with corresponding respiration rates in the summer microcosms indicated a significant linear re-

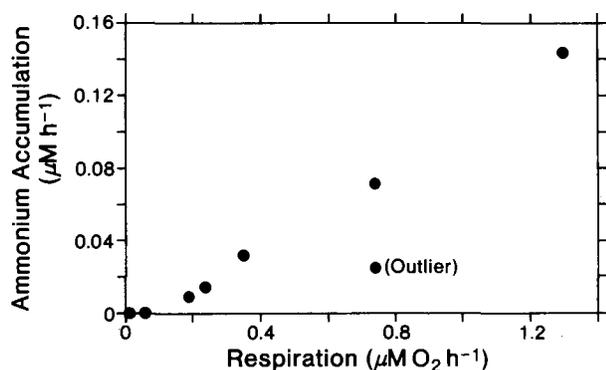


Fig. 5. Relationship of ammonium accumulation rate to respiration rate for the various bottle experiments at different sites in summer.

relationship between the two processes (Fig. 5). Excluding one outlying value (from the filtered treatment at the 16.7‰ station), the correlation coefficient for this comparison was $r = 0.996$ for the relationship: NH_4^+ accumulation = $-0.0085 \mu\text{M NH}_4^+ + 0.114 \mu\text{M O}_2 \text{ h}^{-1}$. The inverse of the slope of this relationship yielded a ratio of 8.76 moles O_2 removed per mole of ammonium accumulated, that is, an O:N atomic ratio of 17.5:1.0.

BACTERIAL COMPONENT OF ORGANIC MATTER DEGRADATION

Integrated estimates of bacterial production rates provided independent estimates of carbon and nitrogen turnover by bacteria in the microcosms (Tables 2–4). In contrast to the community respiration or ammonium accumulation rate results, integrated estimates of bacterial carbon production did not show a consistent pattern of higher rates of production in unfiltered water samples than in the filtered samples (Tables 2 and 3). These results suggest that most bacterial production was supported by dissolved organic matter rather than by particulate materials. As with respiration and ammonium accumulation rates, bacterial production was highest in plume waters of intermediate salinities.

Dissolved amino-acid turnover rates, measured only on the winter cruise (Fig. 6), were highest in the river and decreased with increasing salinity in the plume. This result implies that the river had greater organic carbon demand than the plume if DON composition and isotope dilution are assumed to be comparable at all sites. Similar decreases were observed with increasing salinity when amino-acid turnover rates were normalized for bacterial numbers (Fig. 6b). These results suggest that bacteria had a greater demand for amino acids in the river than in the plume. If amino acids are

TABLE 4. Calculated community ammonium regeneration rates and of bacterial nitrogen turnover rates in summer dark-bottle experiments. Community ammonium regeneration rates were calculated from the mean net ammonium accumulation rates by multiplying by a factor of 1.56 (assuming a nitrogen conversion efficiency of 0.36). Bacterial nitrogen turnover rates were calculated from carbon-based growth rates (Table 1) by assuming a C:N ratio of 5.15 (Goldman et al. 1987) and multiplying this rate by 2.78 ($=0.36^{-1}$) to account for respiration losses.

Station Salinity (%)	Treatment	Mean NH_4^+ Regeneration Rate ($\mu\text{M NH}_4^+ \text{ h}^{-1}$)	Bacterial-N Turnover Rate (BNTR) ($\mu\text{g atom N h}^{-1}$)	Ratio BNTR: $\text{NH}_4^+ \text{ Reg}$
16.7	Whole	0.221	0.060	0.27
	Filtered	0.039	0.057	1.46
17.4	Whole	0.050	0.033	0.66
	Filtered	0.015	0.036	2.40
23.1	Whole	0.111	0.055	0.50
	Filtered	0.022	0.015	0.68

representative of natural labile DON, bacteria in the river and nearshore plume may have had the greatest DON demand of the sites examined in winter.

Excluding data from the summer bluewater station, calculated "bacterial growth efficiency" in the filtered treatments, ranged from 10% to 26% [mean = $19\% \pm 2(\text{SE}, n = 7)$] based on TdR incorporation and from 9% to 42% [mean = $27\% \pm 4 (n = 7)$] based on Leu incorporation. Values

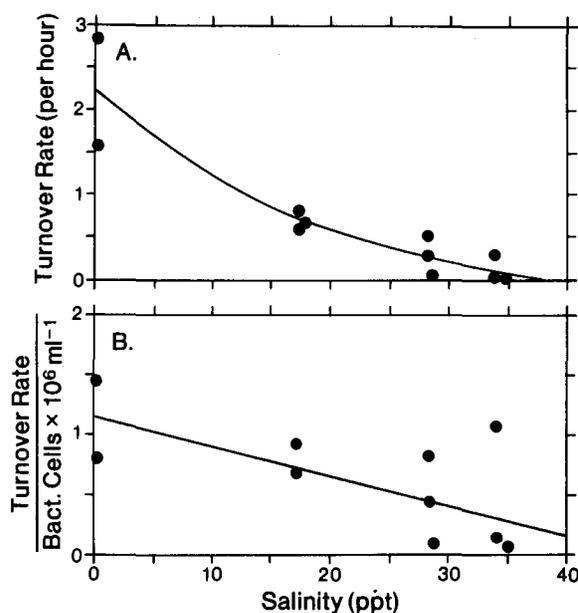


Fig. 6. A. Amino-acid turnover rates at different sites in the Mississippi River plume region during the February cruise. B. Amino-acid turnover rates specific to bacterial numbers. Turnover rates divided by bacterial abundances (cells ml^{-1}) to obtain y -values.

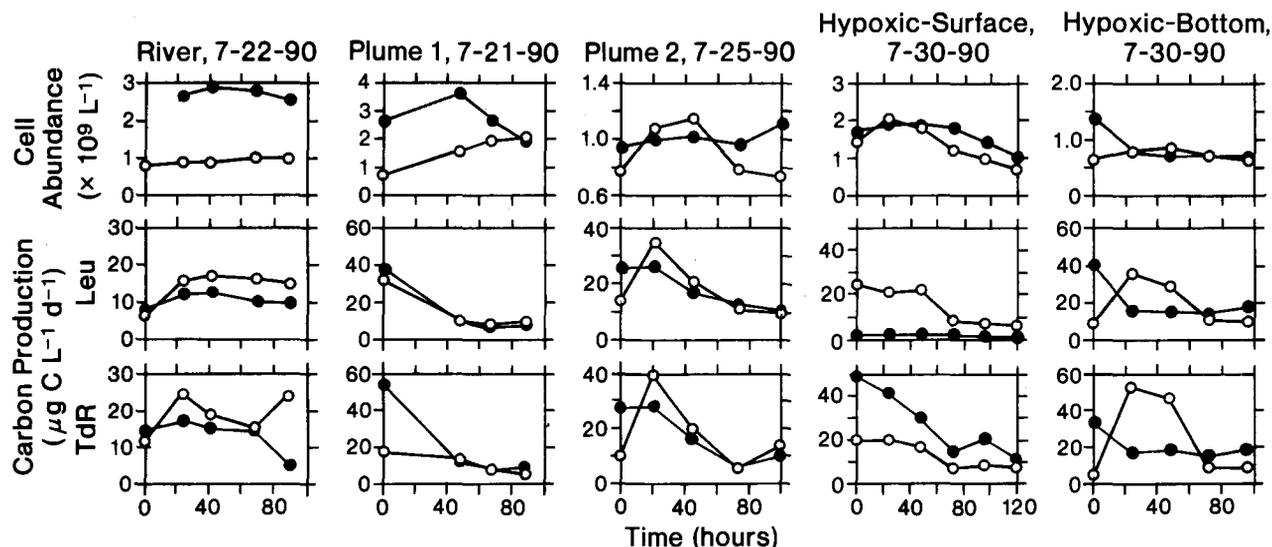


Fig. 7. Bacterial abundances and growth rates determined by Leu uptake and TdR uptake in the summer bottle experiments. Closed circles represent data from unfiltered treatments. Open circles represent data from filtered treatments.

at the summer bluewater station were not included in these calculations because the respiration rates were near the limit of detection for these samples.

KINETICS OF BACTERIAL GROWTH AND ORGANIC MATTER DEGRADATION RATES AFTER REMOVAL OF SAMPLES FROM LIGHT

Although bacterial abundances did not show predictable patterns of change during the incubations at the respective sampling sites, bacterial production rates showed site-specific patterns, particularly in the summer (Figs. 7 and 8). The two

methods of measuring bacterial production (TdR and Leu uptake) generally yielded similar results when expressed as carbon production ($\mu\text{g C l}^{-1}\text{d}^{-1}$) (Figs. 7 and 8). Bacterial production rates in some river and high-salinity waters were relatively constant during the course of the incubations (Figs. 7 and 8). However, in unfiltered mid-salinity summer plume waters where phytoplankton are most abundant (Lohrenz et al. 1990), bacterial production rates in the unfiltered treatments usually decreased as the incubation progressed. Some of the filtered plume samples showed the same

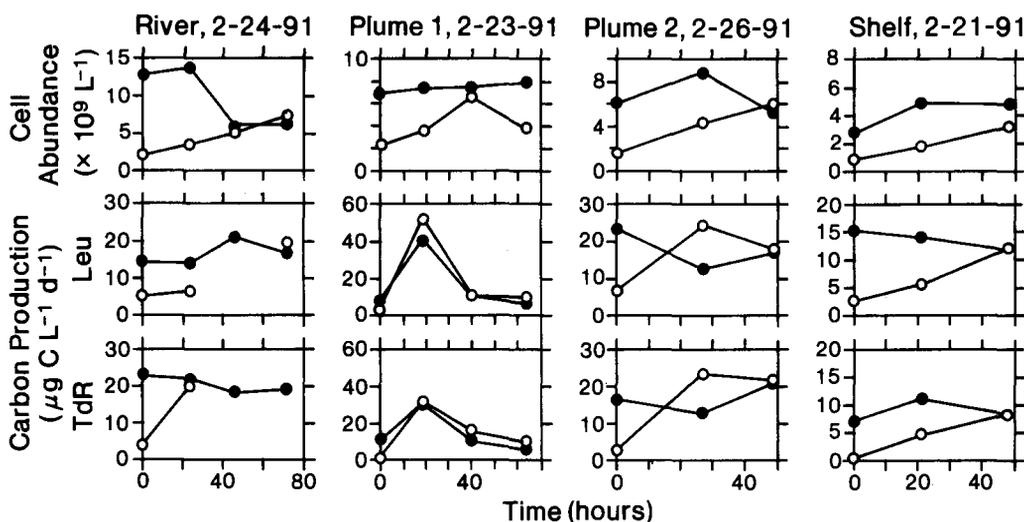


Fig. 8. Bacterial abundances and growth rates determined by Leu uptake and TdR uptake in the winter bottle experiments. Closed circles represent data from unfiltered treatments. Open circles represent data from filtered treatments.

bacterial production patterns as the whole samples, whereas others showed peak production rates on the second day and then gradually decreased to background rates (Figs. 7 and 8). A similar pattern was observed in one of the winter unfiltered plume treatments (salinity 17.3‰; Fig. 8).

Discussion

SPATIAL PATTERNS OF ORGANIC MATTER DEGRADATION RATES

All of the heterotrophic indicators that we examined suggested that water-volume-specific organic-matter degradation rates were higher in the mid-salinity surface plume waters, where phytoplankton production rates are relatively high (Lohrenz et al. 1990, 1992), than at other sites where primary production rates are low (Tables 2–4; Fig. 4). This observation agrees with previous results coupling nutrient remineralization and organic matter degradation with photosynthesis (e.g., Harrison 1978; Kirchman et al. 1991) and is consistent with the positive correlation observed between phytoplankton production and bacterial production in various aquatic environments (Cole et al. 1988). Primary production rates are likely low in the river and deep plume waters due to light limitation and in high-salinity surface waters because of limited nutrient supply rates. On the other hand, production rates are high in the near-river plume because high concentrations of nutrients from the river are delivered into regions of relatively low turbidity (Lohrenz et al. 1990). Our results are consistent with the idea that both heterotrophic bacterial production rates (Chin-Leo and Benner 1992) and nutrient regeneration rates (Cotner and Gardner 1993) are influenced by phytoplankton production of organic material in the near-river plume, particularly in the summer. For example, high rates of respiration and net accumulation of inorganic endproducts in bottles from plume stations suggest greater availability of labile organic matter at these stations than in the river and high-salinity waters (Tables 2 and 3; Fig. 4). However, in the winter when primary and heterotrophic bacterial production rates in the plume are low relative to summer rates, organic matter delivered from the river may support a significant fraction of bacterial activity (Chin-Leo and Benner 1992). This conclusion was supported by the observation that winter amino-acid turnover rates progressively decreased from the river to offshore regions (Fig. 6).

Accumulation rates for ammonium (Fig. 3) were highest at the mid-salinity stations. The relatively low rates of ammonium accumulation at the other stations may reflect low concentrations and fluxes

of organic nitrogen substrates. Temperatures were ideal for microbial growth at all of the sampling sites particularly in summer (Tables 2 and 3). Alternatively, low (or negative) net rates of ammonium accumulation could reflect a greater microbial demand for inorganic nitrogen, particularly ammonium, than was observed at the mid-salinity stations.

The samples from high-salinity stations (e.g., bluewater station, and bottom water from the hypoxic region) did not accumulate any ammonium during the course of our incubations. This result indicates either that ammonium was not produced in these waters or that any ammonium produced in the bottles was quantitatively removed by microbes as rapidly as it was produced. If bacteria use organic substrates with a high molar C:N ratio (i.e., greater than 10:1) and have growth efficiencies of about 50%, they would be net consumers of ammonium (Goldman et al. 1987). However, $^{15}\text{NH}_4^+$ isotope dilution studies with added concentrated bacteria indicated that the cell-specific ammonium regeneration rates were relatively high in offshore waters and that the C:N ratio of labile substrates must therefore be low (Cotner and Gardner 1993). An alternative explanation for no ammonium accumulation at the high-salinity sites is that autotrophic nitrifiers may have used ammonium as an energy source and quantitatively removed it from solution. Unfortunately, we were unable to obtain accurate estimates of nitrate in these bottle experiments, but other short-term (24 h) dark incubations with 1-l bottles in May 1992 showed increases in nitrate concentrations that corresponded with ammonium uptake at a plume station with a salinity of 27‰ (personal communication, D. Pakulski, Marine Science Institute, University of Texas at Austin, Port Aransas, Texas). This result suggests that water-column nitrification can occur at relatively high-salinity sites in the plume.

Comparison of ammonium regeneration rates to respiration rates in the same bottles provided information about the composition of the organic material that was mineralized. A ratio of moles of O_2 consumed to moles of ammonium regenerated similar to that predicted by the Redfield formula for marine organic-matter oxidation (i.e., molar ratio of 6.63, Jahnke et al. 1982) would support the conclusion that the organic matter being mineralized had the elemental composition of marine-derived organic material rather than that of terrestrially-derived riverine organic material. A ratio greater than the Redfield ratio could indicate that either the mineralized organic material had a high C:N ratio, as expected for aged or terrestrially-derived riverine organic matter (Sharp 1983; Ertel et al. 1986), or that a disproportionate amount of the

nitrogen being regenerated was reincorporated into bacterial biomass or converted to other forms such as nitrate. Not including the outlier, our summer data showed a very high correlation ($r = 0.996$) between ammonium accumulation rate and respiration among the respective bottle experiments. The ratio of moles O_2 consumed to moles ammonium regenerated, calculated from the inverse slope of the regression line, was 8.76, a value remarkably close to the Redfield ratio of 6.63 (Fig. 5). This result supports the idea that the organic matter being recycled in the plume region in the summer was mainly relatively fresh marine organic material. Similarly, chemical and stable isotope studies indicated a marine origin for much of the DOC and DON in the mid-salinity region (Benner et al. 1992). The relatively low net ammonium accumulation rate represented by the outlier, and the absence of any ammonium accumulation in the two experiments with the lowest respiration rates (Fig. 5), probably resulted from partial or complete nitrification of regenerated ammonium. However, the close adherence to Redfield stoichiometry observed in other treatments indicated that nitrification was not a major process in most of the mesocosm experiments. We did not do a similar comparison for winter treatments because most ammonium accumulation rates were low and not linear with time.

ROLE OF BACTERIA IN ORGANIC MATTER DEGRADATION

The relative importance of the bacteria-size fraction compared to larger organisms in mineralizing organic nitrogen depended on the site of sampling. Ammonium accumulation rates in bottles containing filtered water were similar to those for the unfiltered treatments in the river and offshore stations (Fig. 3). In contrast, the $<1\text{-}\mu\text{m}$ size fraction accounted for only a relatively small fraction (20–25% in the summer) of total ammonium accumulation at the plume stations (Fig. 4). However, bacterial growth rates were similar in both treatments (Table 2). These results suggest that microzooplankton or mesozooplankton may have accounted for a substantial portion of total nitrogen remineralization in water from the mid-salinity plume sites where primary production was relatively high. Grazing by microzooplankton and mesozooplankton is a major process responsible for removing phytoplankton in this region (Dagg and Ortner 1992; Fahnenstiel et al. 1992). Interestingly, our results on the relative importance of bacteria to regeneration rates at the various plume sites differ from results of isotope dilution studies of bacterial-specific regeneration rates in incubators under natural light. In those experiments, bac-

teria contributed the highest percentage of total remineralization at one of the plume sites (about 50% at a site with salinity of 28‰; Cotner and Gardner 1993). A plausible explanation for this apparent difference is that the relative contribution of bacteria to total remineralization was enhanced by organic substrates that were released by phytoplankton in the presence of light but not to the same degree in the dark.

We compared bacterial nitrogen turnover estimated by ammonium accumulation in the filtered bottles to that estimated from bacterial growth rates. We also used the numbers obtained from bacterial growth rates to estimate the relative importance of bacteria to total nitrogen turnover in our unfiltered treatments (Table 4). To make these comparisons, we assumed that the mineralized organic matter had an atomic C:N ratio of 6.6 (i.e., Redfield composition, as implied from the respiration rate: ammonium accumulation rate ratio, Fig. 5) and that 0.64 of the ammonium assimilated by bacteria or other heterotrophs was remineralized, with the rest being incorporated into biomass (i.e., a nitrogen biomass conversion efficiency of 0.36). The value of 0.64 was derived by comparing the dark ammonium-accumulation rate (this study) to the total ammonium regeneration rate (determined by $^{15}\text{N-NH}_4^+$ isotope dilution experiments; Cotner and Gardner 1993) obtained at the same site (mid-salinity station in the winter cruise). The nitrogen conversion efficiency of 0.36 appears reasonable as it is near the middle of the range of values expected for bacteria growing on organic substrates having C:N atomic ratios of 6–7 (Goldman et al. 1987).

Bacterial nitrogen turnover rates were calculated from the carbon-based bacterial growth rates (Table 2) by assuming a bacterial C:N ratio of 5.15 (for bacteria growing on substrates with a C:N ratio of about 6; Goldman et al. 1987) and multiplying the nitrogen-based growth rates by 2.78 ($=0.36^{-1}$) to account for respiration losses. If the above assumptions are correct, the ratio of bacterial nitrogen turnover:total ammonium regeneration rate should theoretically fall between zero (no NH_4^+ regeneration by bacteria) and 1.0 (all NH_4^+ regeneration done by bacteria). Comparison to these two independent measurements of nitrogen turnover rates at three stations, where data for both bacterial growth rates and ammonium accumulation rates were available, yielded ratios ranging from 0.27 to 2.4 (Table 4). The mean ratios were 1.51 (SE = 0.50) for the filtered treatments and 0.48 (SE = 0.11) for the whole water treatments. This comparison suggests that bacterial nitrogen turnover rates calculated by the two independent methods were not significantly different for the fil-

tered treatments. If the values are realistic, they suggest that bacteria could have accounted for essentially all of the heterotrophic nitrogen turnover in the filtered treatments but only approximately one-half of the total nitrogen turnover in the unfiltered plume water treatments. Similarly, bacteria accounted for up to 50% of total ammonium regeneration, as determined by isotope dilution experiments, in plume waters (Cotner and Gardner 1993).

KINETIC PATTERNS OF BACTERIAL PRODUCTION RATES AND END-PRODUCT ACCUMULATION

The patterns of bacterial production and remineralization rates over time, during incubation in the dark, provide insight about the nature and sources of the labile organic material being mineralized. If bacterial production and the remineralization process are directly coupled to primary production on a short time scale, it is reasonable that heterotrophic rates would decrease over time of incubation because exudation of organic matter by growing phytoplankton would decrease in the dark. Thus, if abundances are stable, measurable decreases in bacterial production rates over time, as we observed for the unfiltered summer mid-salinity plume samples, support the hypothesis that a significant portion of bacterial production was derived directly from photosynthetic production. On the other hand, relatively constant bacterial production rates over time, as observed for some river and offshore waters, suggest that bacterial production was not as directly coupled to primary production at these sites as it was in the mid-salinity plume waters in summer.

In most of the filtered treatments (and in one winter unfiltered treatment) from mid-salinity plume waters, bacterial growth rates were initially low but peaked after about 1 d of incubation (Figs. 7 and 8). Some bacteria as well as most of the bacterial grazers were probably removed by the filtration-dilution step. In addition, increased concentrations of labile organic substrate were probably released and made available during filtration (Fuhrman and Bell 1985; Kirchman et al. 1989; Keil and Kirchman 1991) or other experimental manipulations. Thus, it is reasonable that community bacterial growth rates in the filtered samples were initially quite low but increased over time in response to available substrate (particularly in the absence of filtered-out grazers), and finally decreased again as the available substrate was depleted.

In contrast to the patterns observed for bacterial growth rates in the plume waters, we did not generally observe significant decreases in accumula-

tion rates for ammonium (or other endproducts) over time in any of the treatments. The slopes of ammonium accumulation versus time were either linear, not significantly different from zero, or increased after the first or second day (Fig. 3). We did not sample these mesocosms frequently enough to determine kinetic patterns for O_2 and CO_2 , but other 24-h dark-bottle experiments in the same region during May 1992 did not indicate significant rate changes in respiration or inorganic end-product accumulation rates over time of incubation (personal communication, D. Pakulski).

This apparent inconsistency between patterns of bacterial growth rate measurements and of mineralization endproduct accumulation rates may indicate that bacterial growth is decoupled from the mineralization process. For example, animals consuming particles apparently accounted for a relatively large fraction of nutrient remineralization in the unfiltered samples. However, animal excretion should not have been a major factor in the filtered treatments where bacterial growth rates also decreased after an early peak (Figs. 7 and 8). Another reasonable explanation for the discrepancy is that the quality as well as quantity of labile organic substrates decreased over time in the dark. As the "metabolic quality" of available organic substrates decreased, the bacteria and other heterotrophs may have responded by growing less efficiently (Chin-Leo and Kirchman 1990). If the decreased cell growth was compensated by less efficient use of comparable quantities of organic substrates for biomass production or if the C:N ratio of the substrates decreased, the ratio of cell growth to respiratory nutrient excretion would decrease over time. With less efficient use of nutrients for growth, proportionally more endproducts could be released, compensating for the lower growth rates, and apparently constant rates of respiration and nutrient regeneration could result. In agreement with this suggested scenario, when surface (light) and deep (dark) waters were fortified with ^{15}N -labeled amino acids, a larger fraction of the assimilated ^{15}N was recovered as $^{15}N-NH_4$ in the deep samples (90%) than in the near-surface samples (48%; Gardner et al. 1993). The conversion of dissolved free-amino-acid nitrogen to biomass was apparently less efficient when other sources of organic substrate (e.g., from phytoplankton release) were not available.

CONCLUSIONS

The dark-bottle experiments provide insight into the chemical nature, biological reactivity, and potential sources of labile organic materials in water from the river and from different regions of the Mississippi River plume. Advantages of this ap-

proach include the isolation of heterotrophic processes from autotrophic ones and the ability to examine several heterotrophic indicators (respiration rates, accumulation rates of inorganic mineralization products such as CO_2 and NH_4 , and bacterial growth rates) simultaneously in the same enclosures. A disadvantage of the dark-bottle approach is that reaction rates are not necessarily the same as rates in nature because of enclosure effects (Ferguson et al. 1984) and the changes in foodweb structure caused by experimental manipulations such as filtration (Fuhrman and Bell 1985; Kirchman et al. 1989; Keil and Kirchman 1991) and dark incubations. However, when results from these experiments were compared with complementary data on primary production (Lohrenz et al. 1990, 1992), on-site bacterial production rates (Chin-Leo and Benner 1992), community nitrogen regeneration rates (Cotner and Gardner 1993), and organic matter composition (Benner et al. 1992) in the same region, a more complete understanding of heterotrophic organism dynamics in the Mississippi River plume emerges.

Horizontal spatial and temporal patterns in heterotrophic activity observed in these studies indicate that heterotrophic process rates are strongly coupled with phytoplankton processes particularly in the summer. Depth profiles of heterotrophic process rates (Chin-Leo and Benner 1992; Gardner et al. 1993) also indicate that bacterial growth rates and organic matter mineralization rates are highest in the euphotic zone where phytoplankton are most abundant. The excellent agreement that we observed between O:N endproduct ratios and the Redfield formula for fresh marine organic matter (i.e., phytoplankton) oxidation provides evidence for close coupling between phytoplankton production and remineralization processes. Interestingly, the dark-bottle experiments suggested that bacteria accounted for a smaller portion of total respiration or ammonium accumulation in the mid-salinity plume than in regions where phytoplankton production was relatively low. Thus, the relative role of particle consumers in organic matter degradation appeared to increase more than that of bacteria in regions where phytoplankton production was greatest. However, this observation may have been biased by the fact that experiments were conducted in the dark, where production of dissolved labile substrates by phytoplankton was likely reduced relative to that in natural light. For example, our kinetic studies indicated that bacterial growth rates tended to decrease during dark incubations more in mid-salinity plume samples than they did in the treatments from the other stations. Isotope dilution experiments with $^{15}\text{NH}_4^+$ in lighted incubators indicated that bacterial-cell-spe-

cific ammonium regeneration rates were highest in surface mid-salinity plume waters (Cotner and Gardner 1993). Regardless of the composition of the mineralizers, the dominant process controlling the mineralization rates of organic materials in these Gulf of Mexico waters appears to be the rate of supply of labile organic substrates.

Heterotrophic recycling of nutrients in the euphotic zones of the plume region provides a mechanism for primary production to continue after the initial nutrients from the river outflow are depleted (Turner and Rabalais 1991). Strong coupling between heterotrophic and autotrophic processes, as water moves downstream from the Mississippi River outflow, could maintain available nutrients in the euphotic zone and help extend the influence of riverine nutrient inputs beyond that expected for riverine nutrient uptake alone (Cotner and Gardner 1993). The resulting primary productivity in surface waters over an extended region likely contributes to the biological oxygen demand in the relatively shallow coastal regions where hypoxia develops downstream from the Mississippi River outflows (Turner and Allen 1982; Rabalais et al. 1991, 1992).

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