PRIMARY RESEARCH PAPER

Effects of Mississippi River water on phytoplankton growth and composition in the upper Barataria estuary, Louisiana

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Abstract Diversion of river waters to adjacent estuaries may occur during wetland restoration, navigation channel development, or storms. We proposed that diversions of nitrogen- and phosphorus-enriched waters from the river to estuarine waters would result in increased phytoplankton biomass and shifts to noxious or harmful algal blooms. We tested this hypothesis by conducting four seasonal microcosm experiments in which Mississippi River water was mixed at different volume ratios with ambient estuarine waters of three lakes in the upper Barataria Basin, Louisiana, USA. These lakes included two brackish lakes that were in the path of diverted Mississippi River water, and a freshwater lake that was not. The results from the 3- to 8-day experiments yielded a predictable increase in phytoplankton biomass related to nutrient additions from Mississippi River water. The subsequent decreases in the dissolved nitrate + nitrite, soluble reactive phosphorus, and silicate concentrations explained 76 to 86% of the increase in chlorophyll a concentrations in the microcosms. Our experiments showed that cyanobacteria can successfully compete with diatoms for N and P resources even under non-limiting Si conditions and that toxic cyanobacteria densities can increase to bloom levels with increased Mississippi River water

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inputs to ambient waters in the microcosms. Diversions of Mississippi River into adjacent estuarine waters should be considered in relation to expected and, possibly, unexpected changes in phytoplankton communities to the receiving waters and coastal ecosystems.

Keywords Mississippi River diversions · Nutrient enrichment · Phytoplankton community · Toxic algae · Eutrophication

Introduction

Eutrophication is symptomatic of estuaries with relatively long water residence times and nutrient enrichment (Rabalais, 2004). The increased carbon production is often accompanied by a changing phytoplankton community composition that commonly leads to the appearance and blooms of noxious or toxic harmful algae and oxygen-depleted waters (Dortch et al., 1999; Rabalais, 2004). High phytoplankton biomass is well documented in the Barataria Bay estuary bordering the western levee of the Mississippi River (Turner et al., 2019). Similar conditions exist in estuaries east of the Mississippi River during high flow conditions and when river water is diverted into Lake Pontchartrain and other estuaries (Dortch et al., 1999; Turner et al., 2004; Ren et al., 2009; Riekenberg et al., 2015; Bargu et al., 2019). Understanding the dynamic relationships between nutrient enrichment and phytoplankton growth is an important goal to accurately predict, manage, and, perhaps, reverse eutrophication.

Assessing and predicting the consequences of eutrophication can be complicated by the various nutrient quantities and their ratios, the timing of the nutrient loadings, and the complex suite of other environmental factors and trophic interactions in receiving basins (Glibert et al., 2010). The nutrient(s) limiting phytoplankton growth in estuarine and coastal waters, for example, may include N, P, or Si limitation, as well as co-limitation (Turner et al., 1990; Sylvan et al., 2006; Ren et al., 2009; Turner & Rabalais, 2013; Paerl et al., 2016). Furthermore, although there are strong correlations between the concentration of chlorophyll a (Chl$a$) and both total nitrogen (TN) and/or total phosphorus (TP) in

estuaries and coastal waters (Guildford & Hecky, 2000; Hoyer et al., 2002; Nielsen et al., 2002; Smith, 2006; Cloern et al., 2016), caution is needed to define these relationships. The TN and TP in phytoplankton-N and -P, for example, may not be the dominant component of TN and TP in the water column. Even when phytoplankton biomass is subtracted from TN and TP, as in some studies (e.g., Nielsen et al., 2002), microzooplankton may graze substantial amounts of the phytoplankton biomass resulting from nutrient enrichment (Murrell et al., 2002; Wong et al., 2016). Thus, the internal biological interactions and nutrient regeneration vary in different aquatic systems and, consequently, their responses to external nutrient inputs may differ.

The estuarine waters adjacent to the lower Mississippi River are subject to numerous nutrient inputs via natural processes, from human-influenced high nutrient loads, and from human-engineered movement of Mississippi River water through river diversions and siphons. The Davis Pond Diversion (DPD), completed in 1992, and located 190 km above the Mississippi River delta, diverts river water into the upper Barataria Basin (Fig. 1). Moving Mississippi River water into the estuary could aggravate the eutrophication problems in the estuaries, which are already eutrophic to hypereutrophic (Turner et al., 2019). The construction and operation of the DPD was planned to bring fresh water into the upper Barataria Basin (CPRA, 2017). Other diversions are designed to bring more sediment into the Barataria Basin. There will always be much higher nutrient concentrations when either fresh water or sediments from the Mississippi River enter estuarine waters. We know from marsh fertilization experiments that additional N and P leads to marsh loss (Turner, 2011; Deegan et al., 2012) and that additional nutrients to lake microcosms enhanced chlorophyll biomass accumulation and can potentially promote harmful algal blooms (Ren et al., 2009).

Here we report the results from controlled experiments in microcosms (3–7 days) that document the responses of phytoplankton to the addition of mostly nutrient-rich Mississippi River water into the estuarine waters of Barataria Bay. We previously completed a series of nutrient addition experiments to determine qualitatively nutrient limitation on phytoplankton growth in the same lakes (Ren et al., 2009). We discuss here the quantitative effects of River water as a whole and on a seasonal basis as nutrient
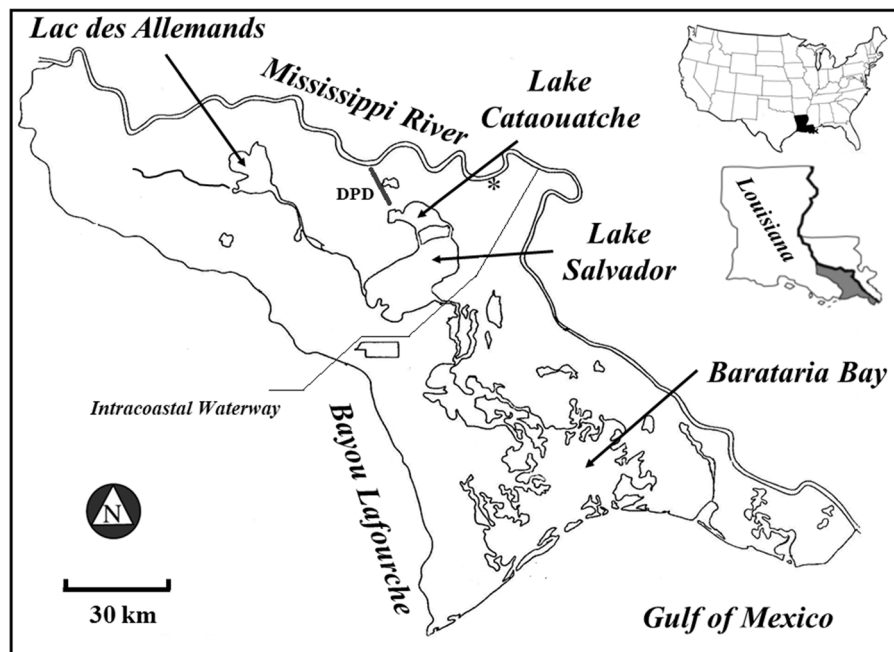
Fig. 1 Map of the study area in the Barataria Bay estuary, Louisiana, identifying the three lakes of concern and location of the Davis Pond Diversion (DPD). The asterisk (*) identifies the location of Mississippi River water collections

concentrations in ambient waters change their concentration and ratios. We hypothesized that the increased River water nutrient loading into three lakes (fresh water to brackish salinities) would increase the growth of phytoplankton and cause a shift in phytoplankton species composition.

Materials and methods

Study area

The Barataria Basin estuary is located in Louisiana's Mississippi River Deltaic Plain. The western margin is bordered by Bayou Lafourche, and the eastern margin is the west bank of the Mississippi River (Fig. 1). The three lakes (average depth 1 to 2 m) are in the upper Barataria Basin and receive nutrient runoff from the surrounding urban and agricultural areas. Lac des Allemands and Lake Cataouatche are freshwater lakes and Lake Salvador is somewhat saltier, but less than 5 psu (see below). A narrow channel connects lakes Cataouatche and Salvador. The water volume of lakes Cataouatche and Salvador is estimated to be about 7.46×10^7 m^3 and 3.96×10^8 m^3, respectively. The

Chl$a$ concentration in Lac des Allemands from 1994 to 2016 varied between 20 to more than 100 μg l^{-1}, identifying it as hypereutrophic, with seasonally dense cyanobacterial blooms (Turner et al., 2019). Lake Cataouatche is moderately eutrophic (average Chl$a$ of 9 to 30 μg l^{-1}), whereas Lake Salvador to the south is the least eutrophic (average Chl$a$ of 6 to 14 μg l^{-1}) among the three lakes (Ren et al., 2009; Turner et al., 2019).

The DPD opened in 2002 and diverted Mississippi River water into Lake Cataouatche, where water flowed into Lake Salvador, and then downstream into the lower Barataria Basin (Fig. 1). Three river diversions occurred during the experiments (Fig. 2). These diversions delivered a daily discharge averaging 1 to 3×10^6 m^3 d^{-1}. The high discharge in January–March 2006 was 1×10^8 m^3 d^{-1}, which could replace 14% of the water in Lake Cataouatche every day, and 2.5% per day for Lake Salvador (Table 1).

Experimental design

Four sets of microcosm experiments were conducted seasonally on lakes Cataouatche and Salvador and Lac des Allemands from May 2005 to February 2006. Lake
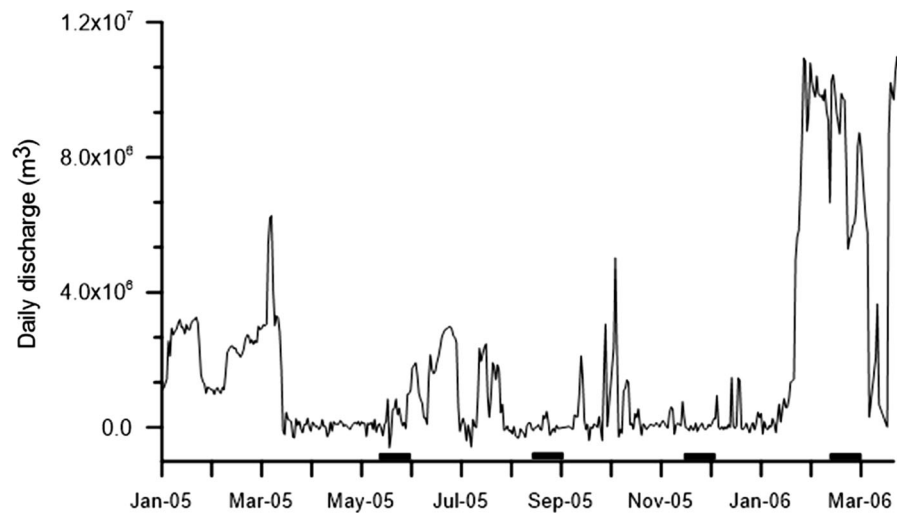
Fig. 2 Davis Pond Diversion (DPD) daily discharge ($m^3 d^{-1}$) at the time of four experiments (indicated with horizontal bars). The time of four sets of experiments: May 16–26, August 15–26, November 28–December 14 of 2005, and February 13–March 1 of 2006

Table 1 Estimate of lake water replacement by DPD discharge per day at the time of four experiments

Lake	Experiment	Experiment duration	DPD discharge ($\times$ 1000 m^3)	Water replacement (per day) (%)
Cataouatche	May05	May 23–26, 2005	370–845	0.5–1.1
	Aug05	Aug. 15–18, 2005	0–18	0–0.02
	Nov05	Nov. 28–Dec. 4, 2005	0–258	0–0.35
	Feb06	Feb. 13–20, 2006	6648–1044	9–14
Salvador	May05	May 16–19, 2005	0–842	0–0.21
	Aug05	Aug. 15–18, 2005	0–18	0–0.004
	Nov05	Nov. 28–Dec. 4, 2005	0–258	0–0.07
	Feb06	Feb. 13–20, 2006	6648–1044	1.7–2.6

water was collected from the middle of each lake. Mississippi River water was collected from the Louisiana Universities Marine Consortium (LUMCON) Audubon Zoo monitoring station (29° 55.260′ N, 90° 8.070′ W) in New Orleans (Fig. 1). An additional 1-l water sample was taken from each station to measure the initial water conditions in the River and lakes. The samples were transported to the LUMCON Marine Center in Cocodrie, Louisiana, within 3 h of collection. The Mississippi River water (RW) was mixed in 10-l polycarbonate carboys with lake water (LW) at 5%, 25%, 50% and 75% dilution to make up 10 l, and then incubated as experimental microcosms. Duplicate microcosms were filled with 10 l of 100% lake water or with 100% River water as lake control (LC) and River control (RC),

respectively. Twelve microcosms were set up for each lake and placed in incubators filled with ambient LUMCON marina water. The carboy temperatures were stabilized by continuously pumping marina water through the holding chambers. The incubators and carboys were covered with screens to reduce light intensity by 50% using the same setup described in Ren et al. (2009). After mixing, water samples were taken from each carboy at 0, 1 day, 2 days, and then every 2 days thereafter until the Chl*a* concentration was depleted.

Temperature and solar radiation

We measured water temperature in the incubators and ambient water every 3 h, and the incident solar

radiation and photosynthetically active radiation (PAR) was recorded by the monitoring station at the LUMCON marine center (http://weather.lumcon.edu/). The light intensity in the holding chamber was measured below the surface water with and without screens using a LI-COR Inc. LI-189 Photometer and LI-193SA Sensor at the beginning of each experiment to ensure that the light intensity was < 50% after screening.

Suspended particulate matter

SPM water samples were filtered through pre-weighed GF/F filters that were pre-combusted at 450°C for 24 h. The filters with filtrate were first dried at 40 to 60°C for 12 h, weighed, and then transferred to an Al cup and combusted at 450°C for 24 h. The filters were weighed again after cooling to room temperature; the weight of the inorganic suspended matter was estimated by subtracting the pre-filtration filter weights. The concentration of organic suspended matter was calculated using the weight difference between the dried and combusted filters.

Nutrients

Samples for nutrient concentration measurements were filtered through 0.2-μm pore size polycarbonate filters, and the filtrates were frozen immediately. Inorganic nutrients were analyzed on a Lachat Quick-Chem 8000 Flow Injection Analyzer, using the Lachat Methods approved by USEPA: method 31-107-06-1-B for ammonium, method 31-107-04-1-C for nitrate + nitrite, and method 31-115-01-1-H for soluble reactive phosphorus (SRP), and method 31-114-27-1-C for silicate.

Chlorophyll a

Water samples for Chla analysis were filtered through Whatman GF/F filters. The filters were fixed in 5 ml of a mixed solvent of DMSO (dimethyl sulfoxide):90% acetone (40:60 by volume), and extracted for at least 2 h in the dark (Lohrenz et al., 1999). The extracts were measured on a Turner Model 10 fluorometer before and after acidification with 10% HCl (Parsons et al., 1984). The fluorometer was calibrated for Chla against a dilution series of a chemical supply house Chla standard measured on a spectrophotometer.

Phytoplankton species composition

Phytoplankton samples were preserved in 0.5% (v/v) glutaraldehyde and size-fractionated by filtration onto 0.2-, 3-, and 8-μm polycarbonate filters. The latter two fractions were stained with 0.03% proflavine hemisulfate, and the 0.2- to 3-μm fractions were counted immediately after filtration. The > 8-μm fraction was frozen and counted later. All samples were counted using an Olympus BH2-RFCA epifluorescence microscope equipped with a blue and green excitation light and transmitted light (Dortch et al., 1997; Ren et al., 2009). All algae were identified to the lowest taxonomic level possible and unidentified diatoms were categorized as three size fractions (< 10 μm, 10–20 μm and > 20 μm). The detection limit was approximately 500 to 1000 cells l^{-1}, depending on the volume filtered (2 ml to 25 ml).

Algal biovolume was calculated using microscopic measurements of their dimensions, and a geometric model of algae (Hillebrand et al., 1999). The biovolume of each taxon or category was averaged from 15 or more measurements. Biovolume was used to compare fractions among the whole community. Cell density was used to determine bloom status of a species or changes over time.

Calculation of Chla yield

The yield of Chla (Y) per unit of nutrient decrease (dDIN, dP, and dSi) was calculated based on the equation modified from the one for batch culture in Edwards et al. (2003, p. 1775, Eq. 6):

$$Y = \frac{(X_{\max} - X_{\max\_}lc) - (X0 - X0\_lc)}{(S0 - S0\_lc) - (St - St\_lc)},$$

where Y is the yield of Chla; $X\max$, $X\max\_lc$ are the highest concentration of Chla in the experimental and lake control microcosms (lc), respectively; $X0$, $X0\_lc$ are the initial concentration of Chla in the experimental and lake control microcosms (lc), respectively; St, St_lc are the concentration of DIN, SRP, or Si at the time t when the maximum Chla concentration was reached in the experimental and lake control

microcosms, respectively; and $S0$, $S0\_lc$ are the initial concentration of DIN, SRP, or Si, in the experimental and control microcosms, respectively.

Statistical analysis

Excel software was used to develop linear correlations between the increase of Chl$a$ (Y) and the removal of each respective nutrient DIN, SRP, and Si for each lake. A multiple linear regression model was used to explore relationships between the increase of Chl$a$ (Y) and the uptake of three nutrients (dDIN, dP, and dSi) using the data from all experiments for each lake. The latter analysis was conducted using MATLAB 6.5 where $P < 0.05$.

Results

Delivery of Mississippi River water to adjacent waterways

The DPD discharged intermittently during our experiments (Swenson et al., 2005; Das et al., 2012). In most sampling periods (spring, summer and fall of 2006), the DPD was delivering minimal, if any, fresh water, sediments and nutrients to lakes Cataouatche and Salvador (Fig. 2). The most substantial input of the Mississippi River into adjacent estuarine waters was in early February through mid to late-February, 2006, when the February 2006 experiments were conducted.

Initial lake conditions

The salinity of Lakes Cataouatche and Salvador ranged from 0.2 to 5.0 psu in the four experiments (Table 2). The SPM concentrations were generally lower in Lake Salvador and the Secchi depths were higher in Lake Cataouatche (Table 2). The highest SPM concentration observed in both lakes was in February 2006 when the DPD daily discharge was at the highest during the experiments (Fig. 2). The concentration of SPM and Chl$a$ in Lac des Allemands was highest in May and August 2005 (Table 2) during seasonal expression of eutrophication. The water temperature in the microcosms and the daily peak solar radiation were also higher in spring and summer, and lower in fall and winter (Table 2).

The concentrations of DIN in all three lakes were similar, being lowest in May 2005 (≤ 1 μM) and highest in February 2006 (85 μM, Table 2). The concentration of SRP was generally below 1.0 μM in Lake Cataouatche and Lake Salvador in all seasons, while the SRP concentration in Lac des Allemands varied the most, with the highest in August 2005 (> 6 μM) and lowest in February 2006 (0.4 μM). The initial concentration of Si in the lakes was highest in spring 2005 at ≥ 100 μM, and exceeded 50 μM in all three lakes most of the time.

The initial concentrations of DIN and SRP in the Mississippi River were much higher than in the three lakes during the period of experimentation (Table 2) and generally higher in the lakes when the diversion was operating. This created, in general, higher nutrient concentrations at the beginning of the experiments as the RW replacement proportion increased. The highest DIN concentration in the Mississippi River was in May 2005 (120 μM), which was about 120 to > 200 times higher than that in the lakes. The concentration of Si in the Mississippi River was about 1 to 5 times higher than that in the lakes. However, in summer (August 2005), the concentration of Si in the river water dropped to 1.5 μM, which was lower than that in the lakes (Table 2), and close to the theoretical minimal threshold for Si uptake by phytoplankton (2 μM, Egge & Aksnes, 1992). When Mississippi River water was highest in Chl$a$ concentration, the biovolume fraction of diatoms of the phytoplankton community was at its highest.

The concentration of Chl$a$ at the beginning of the experiments varied seasonally in the lakes and the Mississippi River (Table 2). In general, the Chl$a$ concentration in Lake Cataouatche was higher than that in Lake Salvador. An extremely high Chl$a$ concentration (> 200 μg l^{-1}) was documented in May 2005 in Lake Cataouatche. The Chl$a$ concentration in Lac des Allemands was generally high in August and November, at > 80 and 140 μg l^{-1}, respectively, but was reduced to 17 μg l^{-1} in February (Table 2). The concentration of Chl$a$ in the Mississippi River was generally lower than that in the lakes.

Development of Chl$a$ in microcosms

The time series of Chl$a$ concentration in the control and experimental microcosms are in Fig. 3. The addition of Mississippi River water promoted a higher

Table 2 The hydrological conditions, initial concentration of nutrients (μmol l^{-1}) and chlorophyll a (Chl$a$, μg l^{-1}) of the lakes and Mississippi River, and temperature and solar radiation during the experiments from May 2005 to February 2006

	Salinity (psu)	Secchi depth (m)	SPM (mg l^{-1})	Lake water (initial)				Mississippi River (initial)				Temperature (°C)	Solar radiation (W m^{-2})
				DIN	SRP	Si	Chl$a$	DIN	SRP	Si	Chl$a$		
Lake Cataouatche													
May05	0.2	0.6	5.8	1.0	0.2	20	208	128	1.5	93	6.9	25–30	937–977
Aug05	0.2	0.8	8.7	2.4	0.6	111	25	52	1.9	1.5	61	30–33	939–1007
Nov05	3.0	0.5	19	6.4	0.5	90	29	86	1.9	111	12	15–19	496–709
Feb06	0.3	0.3	84	85	0.5	76	42	88	2.0	112	13	12–19	94–865
Lake Salvador													
May05	0.2	0.9	6.0	0.4	0.2	71	11	119	1.5	104	11	28–32	795–941
Aug05	0.3	1.7	2.3	2.8	0.6	100	15	52	1.9	1.5	61	30–33	939–1007
Nov05	5.0	1.4	4.7	18	0.9	78	12	86	1.9	111	12	15–19	496–709
Feb06	1.4	0.4	53	53	1.0	79	21	88	2.0	112	13	12–19	94–709
Lac des Allemands													
May05	0.1	0.3	68	–	–	–	–	–	–	–	–	–	–
Aug05	0.1	0.3	27	0.2	6.4	193	83	46	1.1	1.4	34	31–33	653–944
Nov05	0.4	0.9	9.1	12	1.2	25	148	98	2.8	83	7.0	10–14	146–680
Feb06	0.23	1.0	8.5	20	0.4	50	17	93	2.7	108	8.7	15–21	261–855

biomass and longer growth period for phytoplankton in the experimental microcosms, even when the initial Chl$a$ concentration was high in lakes, such as in the experiments on Lake Cataouatche starting May 2005, and in Lac des Allemands starting in August 2005 and November 2005 (Table 2). There was a significant linear increase in Chl$a$ concentration with a higher RW/LW mixing ratio in all Lake Cataouatche and Lake Salvador microcosms (Table 3). The Chl$a$ increase (the difference between the initial and peak concentration of Chl$a$) corresponding to a 1% RW replacement volume into ambient water varied from 1.1 to 2.9 μg l^{-1} in the Lake Cataouatche experiments, and 0.95 to 2.0 μg l^{-1} in the Lake Salvador experiments (Table 3). The relationship between Chl$a$ increase and % RW varied in the three Lac des Allemands experiments. In August 2005, the concentration of the Chl$a$ in the 75% RW microcosms started high (> 100 μg l^{-1}) and did not show much increase compared to that in the lake controls (LC), and the relationship appeared to be polynomial. The concentration of Chl$a$, however, increased either linearly or exponentially with an increase in the RW replacement time in November and February, respectively (Table 3).

Inverse correlation between Chl$a$ increase and nutrient decrease

The nutrient concentration in microcosms decreased gradually over the 3- to 7-day incubation period (Figs. S1–S3), mirroring the increase in Chl$a$ concentration (Fig. 3). In general, the Chl$a$ concentration decreased after the DIN or SRP, or both were exhausted in the microcosms (Figs. S1–S3). There was a significant linear correlation between the increase in Chl$a$ concentration (Y) and the decrease of nutrient concentrations (dDIN, dP, and dSi). The Chl$a$ increase in Lake Cataouatche and Lake Salvador was significantly related to dDIN and dSi, but less significantly related to dP (Table 4). Among the three nutrients, dDIN was significantly correlated to dSi, but less so with the dP in Lake Cataouatche, whereas dDIN in Lake Salvador was more significantly coupled with dP than with dSi (Table 4). For Lac des Allemands, when data from all the experiments were considered, the increase of Chl$a$ was significantly correlated with decreasing dP, but had a weak or insignificant linear correlation with dSi and dDIN. When only the November 2005 and February 2006 data were considered, the linear correlation between
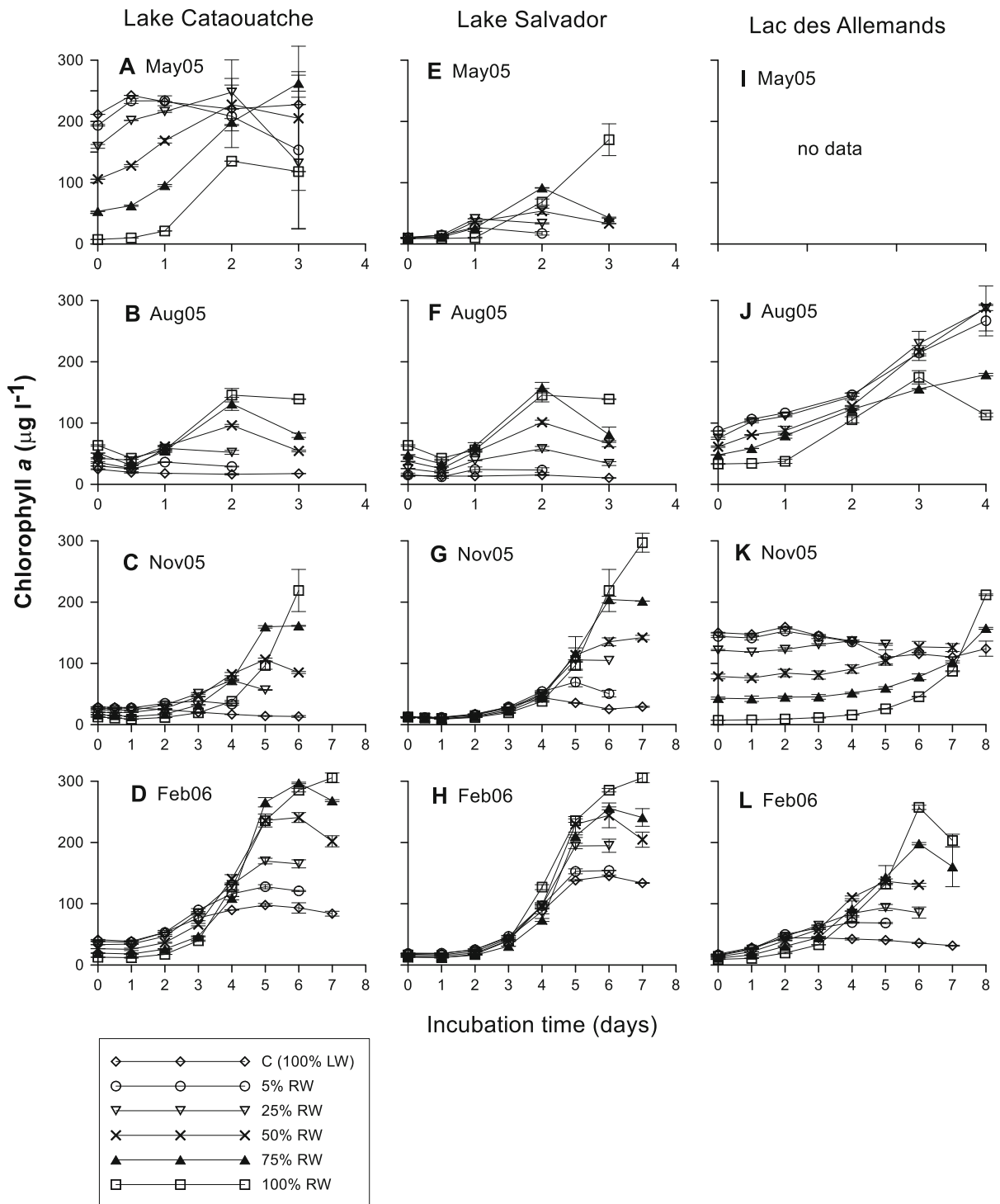
Fig. 3 The development of Chl*a* concentration (μg l^{-1}) in the four experiments mixing different with Lake water of Cataouatche, Salvador and des Allemands. *LW* lake water, *RW* Mississippi River water

Table 3 Correlation of Chl*a* increase with the percentage of Mississippi River water mix in the experiments of Lake Cataouatche, Lake Salvador and Lac des Allemands

Lake	May05	Aug05	Nov05	Feb06
Cataouatche	$Y = 2.3 \times X + 28$ ($R^2 = 0.97$)	$Y = 1.1 \times X - 3.5$ ($R^2 = 0.99$)	$Y = 1.9 \times X - 3.2$ ($R^2 = 0.99$)	$Y = 2.9 \times X + 66$ ($R^2 = 0.99$)
Salvador	$Y = 0.95 \times X + 5.4$ ($R^2 = 0.95$)	$Y = 1.4 \times X - 1.4$ ($R^2 = 0.99$)	$Y = 2.0 \times X + 38$ ($R^2 = 0.97$)	$Y = 1.6 \times X + 131$ ($R^2 = 0.96$)
des Allemands	No data	$Y = 1.5 \times X + 160$ ($R^2 = 0.82$)	$\ln(Y) = 0.04 \times X + 2.0$ ($R^2 = 0.98$)	$Y = 2.0 \times X + 32$ ($R^2 = 0.98$)

X percentage of river water (%RW), R^2 coefficient of correlation

Table 4 Linear correlation coefficient (R) between the Chl*a* yield (Y) and the nutrient decrease (dDIN, dSi, dP) from experiments on the three lakes

Lake	Y	dDIN	dSi	Equation
Lake Cataouatche				
dDIN	0.86	n.s.	n.s.	$Y = 2.4 \times d\text{DIN} - 12$
dSi	0.70	0.69	n.s.	$Y = 1.0 \times d\text{Si} - 5.4$
dP	0.52**	0.50**	0.64*	$Y = 88 \times d\text{P} + 22$
Lake Salvador				
dDIN	0.86	n.s.	n.s.	$Y = 2.8 \times d\text{DIN} - 13$
dSi	0.79	0.60*	n.s.	$Y = 1.5 \times d\text{Si} + 11$
dP	0.79	0.76	0.71	$Y = 105 \times d\text{P} - 17$
Lac des Allemands				
a. Based on experiments in Aug05, Nov05 and Feb06				
dDIN	0.03[#]	n.s.	n.s.	
dSi	0.51**	0.44[#]	n.s.	
dP	0.81	− 0.36[#]	0.46[#]	$Y = 43 \times d\text{P} + 29$
b. Based on experiments in Nov05 and Feb06 (Aug05 excluded)				
dDIN	0.81*	n.s.	n.s.	$Y = 2.4 \times d\text{DIN} - 25$
dSi	0.84**	0.87	n.s.	$Y = 1.6 \times d\text{Si} + 4.5$
dP	0.88	0.73**	0.81*	$Y = 93 \times d\text{P} - 26$

n.s. no statistics

*$P < 0.01$; **$P < 0.05$; [#]Not statistically significant; others: $P < 0.001$

Chl*a* and dDIN, and those among three nutrients became statistically significant (Table 4, case b).

The multiple regression models described the increase of Chl*a* concentration (Y) in lakes corresponding to riverine nutrient inputs (Fig. 4). The coefficient of determination between the actual and predicted Chl*a* concentration suggested that the change of DIN, SRP and Si (dDIN, dP, and dSi) could explain 82% and 86% of the Chl*a* increase in lakes Cataouatche and Salvador, and Lac des Allemands, respectively (Fig. 4).

Phytoplankton community composition

The major phytoplankton groups in the lakes included diatoms, cyanobacteria, chlorophytes, cryptomonads, and euglenoids. Their biovolume fractions at the beginning of the experiments and at the Chl*a* peak are shown in Figs. 5, 6, 7. The lake phytoplankton assemblages were dominated by cyanobacteria in May 2005, accounting for 98% and 78% of the total biovolume in Lake Cataouatche and Lake Salvador, respectively. The dominant cyanobacterium was *Anabaena* cf. *circinali*s, a N-fixing and potentially toxic taxon. Diatoms, mainly *Cyclotella meneghiniana* and *Aulacoseira granulata*, were dominant in August 2005 and February 2006, and accounted for 50% to 75% of
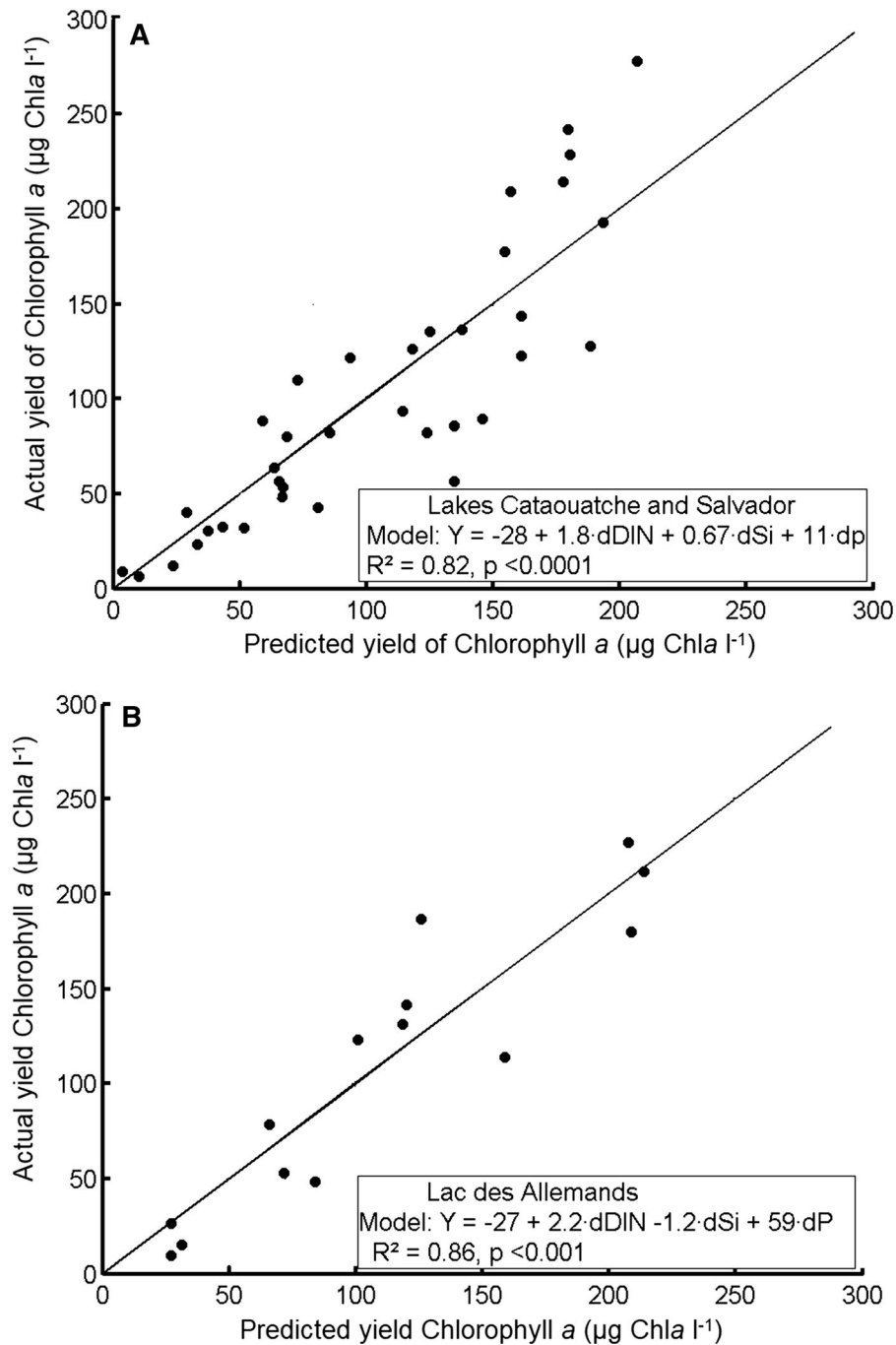
Fig. 4 The prediction models of Chl*a* yield based on multiple linear regression analysis and the comparison with experimental results. *Y*: chlorophyll yield in μg l^{-1}, *dDIN, dSi, and dP* total biovolume (Figs. 5 and 6). An autotrophic ciliate, *Myrionecta rubra* (syn. *Mesodinium rubrum*), was abundant in November 2005, reaching up to 2.5×10^5 decreases of dissolved inorganic nitrogen (DIN), silicate (Si) and soluble reactive phosphate (P), all in μmol l^{-1}. **A** Lakes Cataouatche and Salvador. **B** Lac des Allemands

cells L^{-1}, and accounted for > 50% of the total biovolume of taxa in Lake Cataouatche and Salvador (Figs. 5 and 6). Cyanobacteria taxa were the largest

proportion, by biovolume, of the phytoplankton community in Lac des Allemands at the beginning of all experiments. The August 2005 and November 2005 community was dominated by N-fixing *Anabaena* cf. *circinalis* and *Anabaena annelid*, together with *Microcystis* spp. and accounted for > 80% of total biovolume (Fig. 7). *Microcystis* spp. increased in proportion of the phytoplankton community in February 2006, when it accounted for more than 60% of the total biovolume (Fig. 7). Details of the microcosms from Lake Salvador in November 2005 demonstrated shifts in species and relative proportions of the phytoplankton community in the Mississippi River water additions (Figs. 6 and 8). While the biovolume of cyanobacteria was a small proportion of the phytoplankton community, the densities of potentially harmful cyanobacteria reached or were near bloom densities after 7 days after the 50% RW addition.

Except for the dominant species mentioned above, other frequently detected species in the lakes included diatoms *Skeletonema* spp., *Melosira* spp., *Fragilaria* sp., *Stephanodiscus* sp., *Asterionella* spp. and *Nitzschia* spp. and the cyanobacteria *Anabaenopsis* sp., *Aphanizomenon* sp., *Cylindrospermopsis* sp., and *Raphidiopsis curvata.* Other filamentous cyanobacteria, such as *Planktolyngbya* and *Jaaginema*, and colonial coccoid *Merismopedia* spp., were also frequently detected and sometimes in high densities. Chlorophytes, including *Actinastrum* spp., *Closterium* spp., *Scenedesmus* spp. and *Pediastrum* spp., were commonly present, but did not make up a high biovolume fraction in the initial composition of the lakes. The dominant phytoplankton in the Mississippi River were diatoms in all seasons and accounted for nearly 80% of the total biovolume. The River diatoms included *Cyclotella meneghiniana*, *Stephanodiscus medius,* and *Aulacoseira* spp.

We identified three general phytoplankton community shifts in the experiments based on the initial LW compositions and subsequent changes

Group one consisted of six experiments, including November 2005 and February 2006 of Lake Cataouatche, May 2005, November 2005 and February 2006 of Lake Salvador, and February 2006 of Lac des Allemands. In all these experiments, the initial Chl*a* concentrations were similar in the lakes and the Mississippi River, and the phytoplankton assemblages in the microcosms were dominated by diatoms from the beginning to the end of the experiments (Figs. 5, 6, 7). In November 2005, in Lake Cataouatche and Lake Salvador, *Myrionecta rubra* made up more than 50% of the biovolume biomass, but due to the large cell size, its cell density was actually one to two magnitudes lower than that of diatoms.

Group two consisted of lakes Cataouatche and Salvador in August 2005, featuring high Chl*a* and low Si in the River water at the beginning of the experiments (Table 2). Diatom growth in the microcosms was sustained from high Si in the lake water, and the percent of biovolume as diatoms increased with higher River water replacement (Figs. 5B and 6B). However, because the Mississippi River water contained a low Si concentration, the phytoplankton growth in the 50% and 75% RW microcosms may have undergone Si limitation leading to an increase in the percentage of chlorophytes at the end of the experiment (Figs. 5B and 6B).

Group three was composed of data from the May 2005 Lake Cataouatche (Fig. 5A), and August 2005 and November 2005 Lac des Allemands experiments (Fig. 7B, C), and shared two main characteristics: (1) the initial Chl*a* in the lakes was much higher than that in the River (Table 2); and (2) the lake phytoplankton communities were dominated by cyanobacteria, whereas the Mississippi River phytoplankton was dominated by diatoms. However, the change in nutrients, Chl*a*, and phytoplankton composition differed among the experiments. In the May 2005 Lake Cataouatche experiment, the DIN and SRP were taken up within 1 to 2 days in most microcosms (Fig. S1), while the Chl*a* peak occurred 1 or 2 days after the nutrient depletion (Fig. 3A), and phytoplankton were dominated by cyanobacteria (Fig. 5A). In the August 2005 Lac des Allemands experiment, the concentration of Chl*a* increased gradually to the end of the experiment, but no distinct peak was detected (Fig. 3J), and cyanobacteria were dominant in all microcosms, including the initial LW (Fig. 7B). The increase of Chl*a* was relatively slow in the November 2005 Lac des Allemands experiment when compared to other experiments (Fig. 3K). The diatom fraction increased as the increasing Mississippi River volume percentage brought in Mississippi River diatoms and nutrients (Fig. 7C).
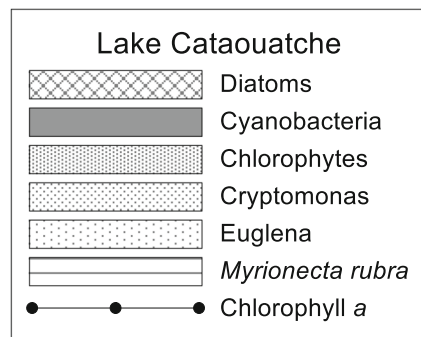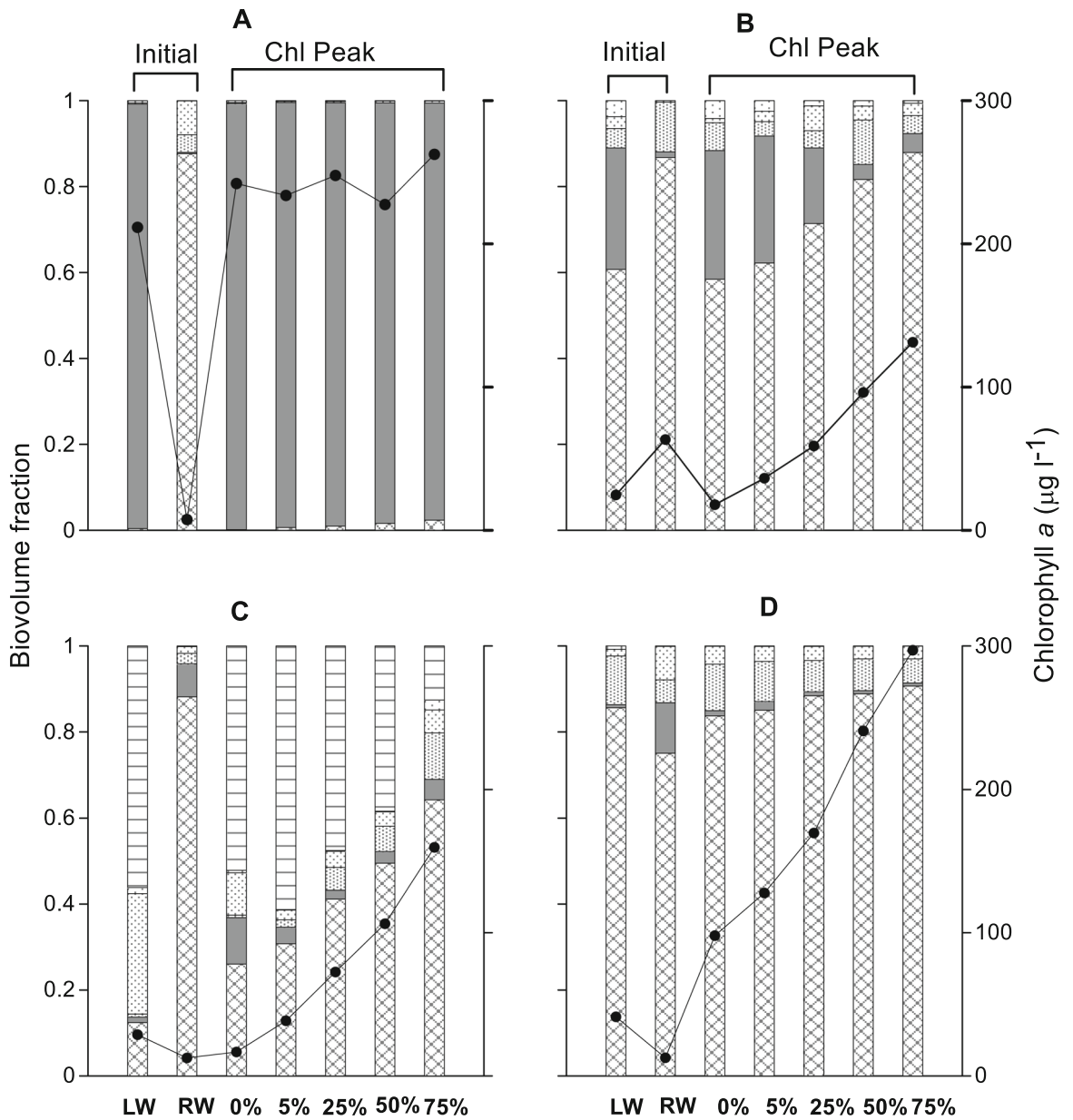
◀ **Fig. 5** Biovolume fractions of major phytoplankton groups and Chl*a* concentration in the initial Lake and River water and the microcosms at the Chl*a* peak in the experiments of Lake Cataouatche in May 2005 (**A**), August 2005 (**B**), November 2005 (**C**), and February 2006 (**D**)

Discussion

The general result from the four sets of microcosm mixing experiments is that introducing Mississippi River water to the upper Barataria Basin will yield a predictable increase in phytoplankton biomass and changes in species composition that will often include toxic cyanobacteria. These robust results must be considered as strong inferences about ecosystem behavior, while acknowledging that there are artifacts inherent to microcosm experiments that do not represent all conditions in the environment.

Chlorophyll *a* yield

The concentration of nutrients in the Mississippi River was usually much higher than in the lakes and provided an important nutrient source for phytoplankton growth in lakes Cataouatche and Salvador, as indicated by the significant linear correlation between the Chl*a* increase, the River/Lake water mixing ratios (Table 3), and the decrease in DIN, SRP, and Si concentrations. Even a small amount of Mississippi River water introduced to these lakes resulted in a significant increase in phytoplankton biomass in the form of Chl*a*. The higher coefficient of determination (R^2) between the Chl*a* increase and *d*DIN compared to *d*P and *d*Si indicated a stronger role for N on the phytoplankton growth compared to SRP and Si. These results are consistent with results from our previous bioassay experiments demonstrating that N was the sole or primary limiting nutrient in these lakes, although N and P co-limitation sometimes occurred (Ren et al., 2009).

The Chl*a* yield was about 2.4 and 2.8 µg Chl*a* µmol N^{-1} for Lake Cataouatche and Lake Salvador, respectively (Table 4). The average yields are well within results from laboratory monoculture and mesocosm studies (1.3-3.2 µg Chl*a* µmol N^{-1}) (Gowen et al., 1992). These values are also comparable to those from the batch culture study by Edwards et al. (2003), which showed that the maximum

Chl*a* yield from available DIN at the bloom peak was 2.35 µg Chl*a* µmol N^{-1}. The bottom-up effects from nutrients are assumed to exceed those from the grazing (top-down) and other stressors (light and temperature were controlled) in controlling the increase phytoplankton biomass (Murrell et al., 2002; Wong et al., 2016). The Chl*a* yield from these microcosm experiments is probably close to the maximum stimulation of phytoplankton growth in the lakes receiving River water from the DPD. The in situ phytoplankton growth, however, is influenced by hydrological and chemical processes, which modulate the potential Chl*a* yields.

Lac des Allemands, while not receiving Mississippi River inputs, is similar to other Mississippi River deltaic plain lakes in being hypereutrophic and subject to nutrient-enriched watershed runoff, or possibly diverted river water in the future. The Chl*a* increase in Lac des Allemands was only linear with increasing proportions of Mississippi River water when the initial lake Chl*a* concentrations were relatively low, such as in February 2006.

Nutrient co-limitation

The significant linear correlation between the increasing Chl*a* concentration and decreasing DIN, SRP, and Si concentrations, and the significant correlations among these nutrients (Table 4), suggests that all three nutrients are essential in regulating phytoplankton growth and community composition in the three lakes. The multiple linear regression models describe the quantitative relationships to predict phytoplankton biomass yield from nutrient concentrations (Fig. 4).

Three nutrient variables (DIN, SRP, and Si) explained 76%–86% of the variations in Chl*a* concentrations in the experimental systems. The lower significance of *d*P in Lake Cataouatche, when compared to Lake Salvador (Table 4), is due mainly to the results from the May 2005 experiment that used Lake Cataouatche water with high Chl*a*, low DIN, and low SRP concentrations (Table 2). After mixing, the SRP was quickly taken up and depleted within the first 24 h in all microcosms, whereas the Chl*a* concentration only peaked after 48 to 72 h. The de-coupling of SRP uptake and Chl*a* increase was observed previously (Ren et al., 2009), and can be explained by the luxury uptake of P that resulted from the cellular P-deficiency in phytoplankton cells. If the May 2005 experiment
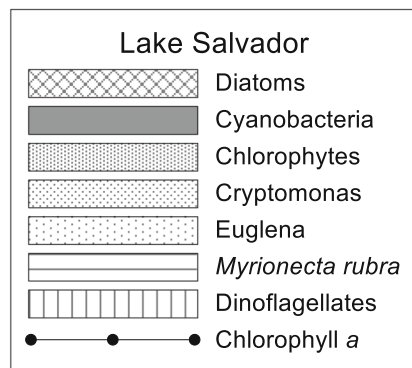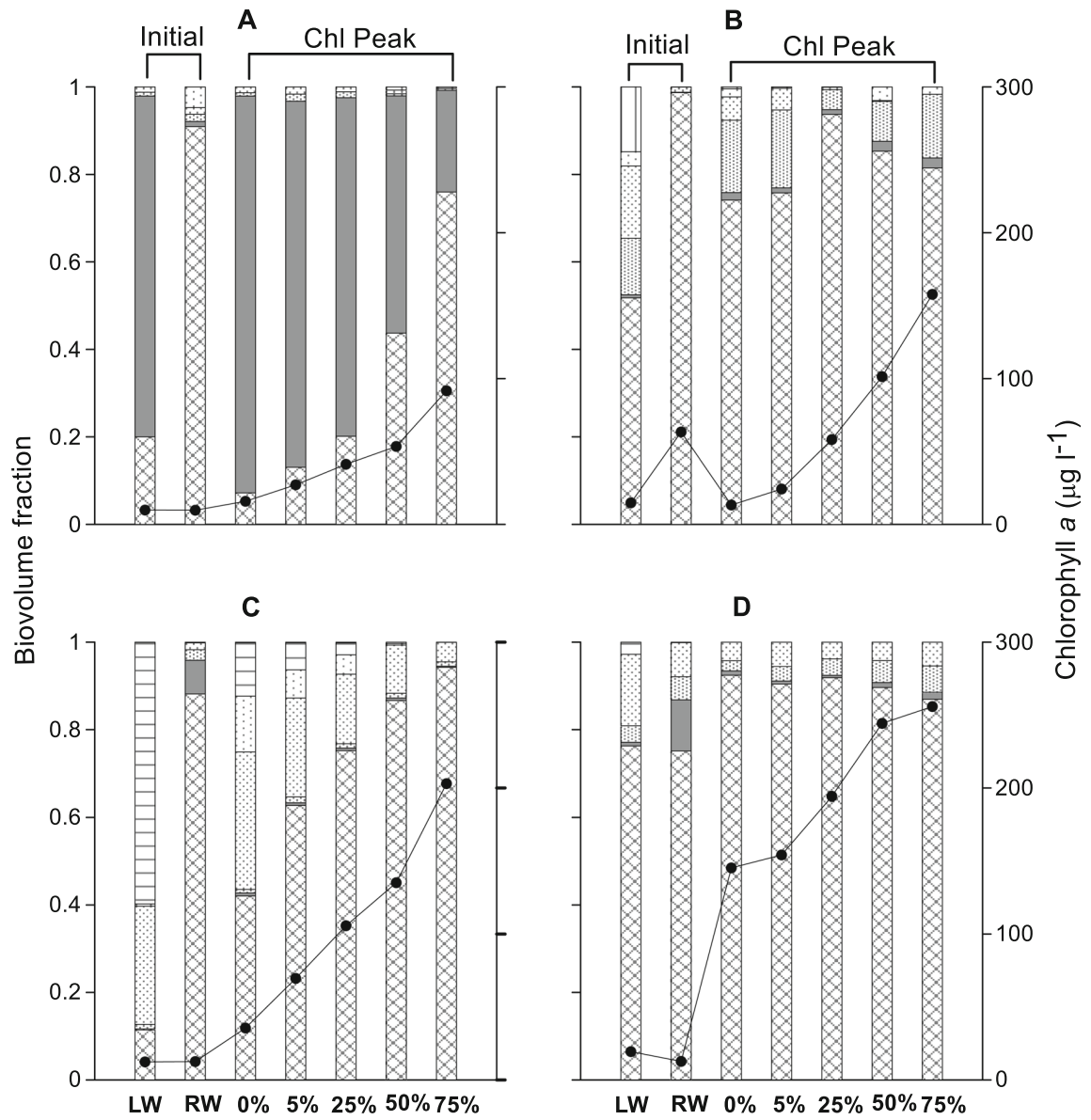
◀ **Fig. 6** Biovolume fractions of major phytoplankton groups and Chl*a* concentration in the initial Lake and River water and the microcosms at the Chl*a* peak in the experiments of Lake Salvador in May 2005 (**A**), August 2005 (**B**), November 2005 (**C**), and February 2006 (**D**)
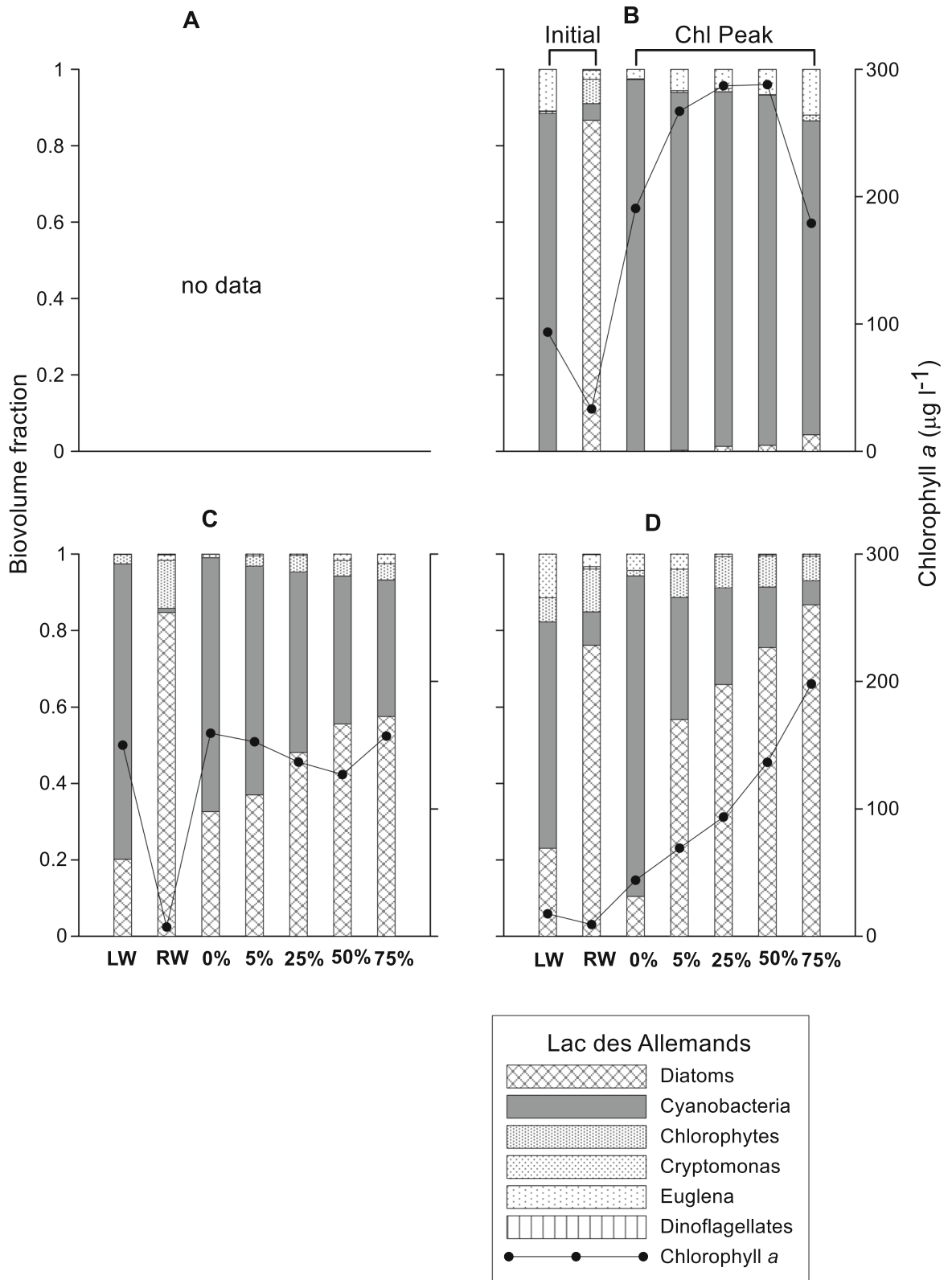
were excluded, the significance of the correlation between the Chl*a* increase and the SRP decrease was higher for Lake Cataouatche ($R^2 = 0.7$, equation not shown).

A comparison of the May 2005 Lake Cataouatche experiment and the Aug 2005 Lac des Allemands experiment is informative with regard to the available DIN and nitrogen-fixation. The phytoplankton community in May 2005 was dominated in density by N-fixing *Anabaena* cf. *circinalis* in Lake Cataouatche (2.9×10^8 cells l^{-1}). But, N-fixation did not seem to occur, because the Chl*a* concentration decreased in the control and was correlated with the decrease of DIN (dDIN) ($R^2 = 0.97$, $n = 5$). By comparison, the Chl*a* concentration in the Aug 2005 Lac des Allemands experiment increased continuously despite the DIN concentration falling below the uptake threshold value and the significant decrease of SRP ($R^2 = 0.74$, $n = 5$) (Figs. 3J, S3), indicating N-fixation during the experiment.

The N-fixing *Anabaena* cf. *circinalis* was the numerically dominant species in the cyanobacterial community in Lake Cataouatche in May 2005 and in Lac des Allemands in Aug 2005 experiments. The ratio of heterocysts to vegetative cells (H:V) for *A.* cf. *circinalis* was similar in both lakes, and varied between 0.033 and 0.035, which is consistent with results from earlier nutrient limitation bioassays (0.02–0.05, Ren et al., 2009). The H:V ratio, however, was low compared to other studies (0.06–0.10 in Lee & Rhee, 1999; and Teaumroong & Innok, 2002), indicating that *Anabaena* in these lakes may have a relatively low N-fixation capacity. It is also possible that N-fixation was inhibited by available ambient inorganic nitrogen in these lakes, especially ammonium (NH_4). Although the concentration of NO_{3+2} was as low as 0.1 μM in both experiments, there was a big difference in the initial NH_4 concentration. The NH_4 concentration was about 0.9 μM in May 2005 Lake Cataouatche. The fast regeneration of NH_4 may have inhibited N-fixation in Lake Cataouatche, as suggested by a previous study in Barataria Bay

(Madden et al., 1988). The initial concentration of NH_4 was as low as 0.1 μM in the Aug 2005 Lac des Allemands experiment, and *Anabaena* and other N-fixing cyanobacteria appeared to have developed the ability to fix nitrogen. As a result, phytoplankton growth showed little correlation with the concentration of DIN in the microcosms. The hypothesis that ammonium may be an inhibiting factor to N-fixation was also confirmed in the Nov 2005 experiment of Lac des Allemands. The initial Chl*a* concentration was high, and cyanobacteria were dominant with 50% of *Anabaena* spp. and 45% of *Microcystis* spp. N-fixation seemed to be absent when the Chl*a* concentration decreased after the depletion of DIN in the lake control microcosms where 80% of initial DIN was ammonium (Table 2).

The availability of dissolved silicate and the proportion to other dissolved inorganic nutrients are key factors regulating phytoplankton species competition and succession (Raven, 1983; Egge & Aksnes, 1992; Milligan & Morel, 2002; Turner et al., 2008). The significant correlation between the increase in Chl*a* concentration and the uptake of Si, DIN, and SRP in both Lake Cataouatche and Lake Salvador (Table 4) suggests that diatoms dominated the new phytoplankton production in the microcosms. When lake diatoms were abundant, e.g., in Aug and Nov 2005, and Feb 2006, the molar ratio of ΔSi:ΔDIN calculated from the experiments was 1.07:1 ($R^2 = 0.79$) for Lake Cataouatche and 1.33:1 ($R^2 = 0.76$) for Lake Salvador. These data are comparable to the results from Brzezinski (1985) who showed that the Si:N ratio of marine diatoms ranged between ∼ 0.8 and 1.2:1 (with a mean value of 1.05) and varied with species composition and light conditions. In general, marine diatoms have less silica content per unit cell than freshwater species (Conley et al., 1989). The dominant diatom species in our study lakes included *Cyclotella meneghiniana*, *Stephanodiscus medius*, *Aulacoseira ambigua*, and *A. granulate*. These species generally have a higher silica content per cell than most marine diatoms, which may explain that the ΔSi:ΔDIN ratios from our experiments are slightly higher compared to previous studies (Brzezinski, 1985). In May 2005, when phytoplankton was dominated by the cyanobacterium *Anabaena* cf. *circinalis*, the ΔSi:ΔDIN ratio decreased to 0.48:1 ($R^2 = 0.70$) and 0.40:1 ($R^2 = 0.81$) in Lake Cataouatche and Lake Salvador, respectively. Using the

A

no data

B

Initial   Chl Peak

C

D

LW RW 0% 5% 25% 50% 75%

LW RW 0% 5% 25% 50% 75%

Biovolume fraction

Chlorophyll *a* (µg l⁻¹)

Lac des Allemands

- Diatoms
- Cyanobacteria
- Chlorophytes
- Cryptomonas
- Euglena
- Dinoflagellates
- ● Chlorophyll *a*

◀ **Fig. 7** Biovolume fractions of major phytoplankton groups and Chl*a* concentration in the initial Lake and River water and the microcosms at the Chl*a* peak in the experiments of Lac des Allemands in May 2005 (**A**), August 2005 (**B**), November 2005 (**C**) and February 2006 (**D**)

ΔSi:ΔDIN ratio from the other three seasons as references (1.05:1 for Lake Cataouatche and 1.33:1 for Lake Salvador), we estimated that approximately half and one-third of the chlorophyll yield was by diatoms. Cyanobacteria successfully competed with diatoms and took up more than half of the riverine DIN for their growth in both lakes in May 2005. However, the Mississippi River water, as in summer 2005, contained a high proportion of diatoms, and a lower Si concentration than the uptake threshold (2 μM, Egge & Aksnes, 1992). The input of Mississippi River water can therefore cause severe Si limitation on diatom growth and promote the growth of non-diatom taxonomic groups in the lakes when under a continuous input of N and P. Furthermore, the high loading of SPM from the Mississippi River may lead to light limitation for most phytoplankton species. Some

cyanobacteria taxa, such as *Anabaena*, *Microcystis*, and *Aphanizomenon*, have selective advantages over light limitation because of their buoyancy that is regulated by gas vesicles (Walsby et al., 1997). This buoyancy provides a competitive advantage in turbid lakes with dense phytoplankton biomass (Chorus & Bartram, 1999). A recent study on whole lake N fertilization showed that the new phytoplankton primary production derived from N enrichment may not be efficiently transferred up to zooplankton, resulting in potential accumulation of phytoplankton biomass (Deininger et al., 2017). For these reasons, the input of Mississippi River water via diversions can support phytoplankton biomass increases and shifts from diatoms to non-diatoms. When the Bonnet Carré Spillway was opened in 1997 as flood prevention for the city of New Orleans and introduced Mississippi River water into Lake Pontchartrain, a bloom developed (maximum Chl*a* concentration of 855 μg Chl*a* l^{-1}) composed of *Anabaena* and *Microcystis* whose toxicity was sufficiently high to lead to a health advisory that closed the lake to recreational contact (Dortch et al., 1999; Turner et al., 2004). Similarly, the high Chl*a* concentration (205 μg l^{-1}) and a dense
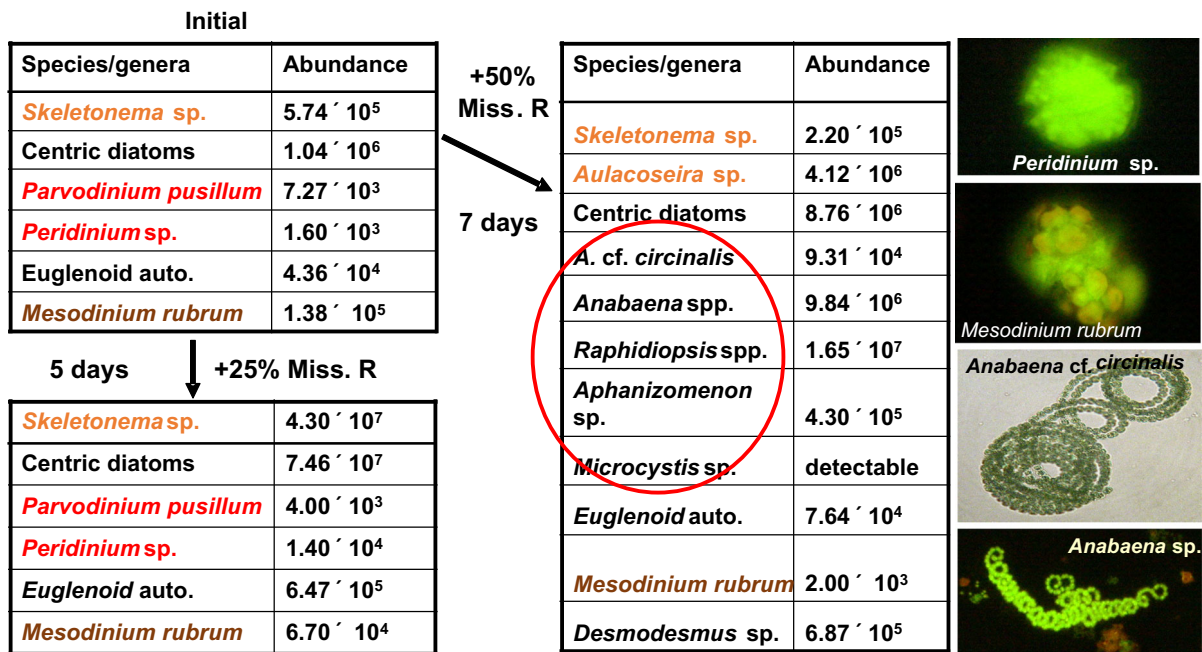

Fig. 8 Changes in phytoplankton taxa and their cell density for Lake Salvador in November 2005. Quantities are cell numbers per liter. Orange fonts represent diatoms; red fonts represent dinoflagellates, and brown fonts represent the autotrophic ciliate

Mesodinium rubrum (syn. *Myrionecta rubra*); the red circle identifies noxious and toxic cyanobacteria that can form large blooms in the lakes. *Note Parvodinium pusillum* (syn. *Peridinium pusillum*)

bloom of *Anabaena* and *Microcystis* observed in May 2005 in Lake Cataouatche were not detected in the long-term monitoring (Turner et al., 2019). Microcystins were detected in April 2005 at 0.3–0.6 mg l^{-1} (protein phosphate inhibition assay; G. Boyer, SUNY-Syracuse, unpublished data).

Delivery of Mississippi River water to adjacent waterways

When the DPD is open, river water is flushed south directly to Lake Cataouatche then south to Lake Salvador. Under high DPD discharge, e.g., in February 2006 (Fig. 2) during our experiments, the water volume of Lake Cataouatche could be completely replaced by Mississippi River water within 8 days, and Lake Salvador within 40 days. As diverted Mississippi River water travels into ambient water, the suspended particulate material falls out, and subsequent improvement in light conditions in the receiving Lake waters coupled with nutrient enrichment can lead to rapid phytoplankton biomass increases within days (Fig. 3). Other diversions of nutrient-rich waters, during flooding and for flood protection, coastal restoration activities, and wastewater treatment, may similarly affect ambient receiving waters (e.g., Dortch et al., 1999; Turner et al., 2004; Riekenberg et al., 2015; Turner, 2011; Bargu et al., 2019; Turner et al., 2019), or not (Bargu et al., 2019).

Conclusion

Our study provides evidence that there are consequences to the phytoplankton community from introducing Mississippi River water into the upper Barataria Basin lakes. The Chl$a$ yield was about 2.4–2.8 µg Chl$a$ µmol N^{-1}, which can be a useful indicator to evaluate changes in phytoplankton biomass in our study area and elsewhere. The multiple linear regression models derived from the experiments provided information on quantifying net phytoplankton production in relation to the input of DIN, SRP, and Si, as predictor variables, which may be specific to the these lakes, and perhaps others. Application of our methodology would be useful in predictive models of responses to shifting water quality in numerous global situations as water resources change qualitatively and quantitatively and are increasingly manipulated by human activity.

Introducing Mississippi River water through the DPD, or other water diversions, can increase the phytoplankton biomass in the receiving lakes and cause increases in density of colonial and often toxic cyanobacteria. The molar ratios of DIN:ortho-P:Si in the Mississippi River are about 16:1:16 (Turner et al., 2007), and is not likely to change nutrient stoichiometry in the lakes in a matter of weeks because the phytoplankton uptake ratio of these three nutrients is close to the riverine ratios (Ren et al., 2009). The Chl$a$ concentration in the lakes in 2005–2006 was significantly higher (Table 2) than that in 1998–2002 (Ren et al., 2009). These high Chl$a$ concentrations and a dense bloom composed of *Anabaena* (2.97×10^8 cells l^{-1}) and *Microcystis* (3.15×10^9 cells l^{-1}) in Lake Cataouatche in May 2005, along with high concentrations of microcystins, could well be a result of the DPD opening.

Diverting Mississippi River water into local lakes and estuaries is proposed as an effective coastal restoration technique (CPRA, 2017), but the water quality in the diverted river may create problems in the ambient receiving waters or downstream, including documented changes in aquatic species quality and quantity (this study). The assumption of wetland restoration plans is that salinity will be reduced and that the introduced nutrients will fertilize emergent marsh growth. However, Turner (2011) demonstrated that excess nitrogen and phosphorus nutrient amendments to salt marshes, while they may stimulate above ground vegetation biomass, result in an increase in soil metabolism and lower root and rhizome biomass that will compromise soil strength and eventually contribute to marsh breakup. Further downstream into coastal waters, the Si:NO$_3$-N ratio in the Mississippi River, for example, shifted from 4:1 in the 1960s to 1:1 by the 1980s (Turner & Rabalais, 1991), which may have affected the phytoplankton community composition in coastal waters (Turner et al., 1998). The diatoms, in particular, appear to have shifted toward smaller and more lightly silicified species (e.g., *Skeletonema* spp. and *Cyclotella* spp.; Rabalais et al., 1996; Dortch et al., 2001), which may further affect higher trophic level organisms in the water column and coastal water food webs (Turner et al., 1998). The more lightly silicified diatoms, included the potentially toxic diatom *Pseudo-nitzschia* spp., remains

increased in sediment cores in coastal waters coincident with increased N loading from the Mississippi River and Si:N ratios < 1:1 (Parsons et al., 2002). Caution is needed, or potential effects considered, when implementing ecosystem recovery plans so that unintended consequences can be identified, weighed before decisions are made and, perhaps, mitigated.

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