Stoichiometry of nitrogen, phosphorus, and silica loads in the Mississippi-Atchafalaya River basin reveals spatial and temporal patterns in risk for cyanobacterial blooms

Todd V. Royer

O’Neill School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana

Abstract

Ratios of nitrogen (N), phosphorus (P), and dissolved silica (DSi) influence how algal communities respond to nutrient loading, and DSi limitation can facilitate cyanobacterial dominance. The indicator of coastal eutrophication potential (ICEP), described previously by other researchers, predicts production by diatoms vs. nonsiliceous taxa based on deviation of nutrient loads from the Redfield ratio of 106C:16 N:20Si (N-ICEP) or 106C:1P:20Si (P-ICEP). The ICEP was calculated for the Mississippi-Atchafalaya River basin, and four subbasins: the Ohio-Tennessee, Missouri, Upper Mississippi, and Arkansas-Red basins from 1979 to 2015. The P-ICEP indicated a stoichiometric imbalance that favored cyanobacteria for all but the Arkansas-Red subbasin. The N-ICEP indicated conditions favorable for cyanobacteria in the Upper Mississippi, Ohio-Tennessee, and the northern Gulf of Mexico. Agriculture is the predominant land use in the Upper Mississippi and Ohio-Tennessee subbasins and these subbasins controlled the stoichiometry of the nutrients delivered to the northern Gulf of Mexico. The imbalance in N, P, and DSi inputs to the Gulf was greatest during spring and early summer, and in most years transitioned to favoring diatoms by August or September. Comparing the 1980–1994 and 2001–2015 periods, there was a significant increase in the P-ICEP for the Upper Mississippi, Ohio-Tennessee, and Missouri subbasins that appeared to arise mainly from increased P loading to surface waters in the those basins. The ICEP revealed patterns in stoichiometry of N, P, and DSi loads among the major tributaries to the Mississippi River, and an increasing risk of cyanobacterial blooms for inland waters in much of the Mississippi-Atchafalaya River basin.

Nitrogen (N) and phosphorus (P) loading to inland and coastal waters relieves nutrient limitation and promotes algal growth. Anthropogenic nutrient loading can alter the composition of algal communities and in severe cases, promote blooms of toxin-forming cyanobacteria or other harmful algal groups. How algal communities respond to N and P loading is determined in large part by the absolute availability of dissolved silicate (SiO₂, hereafter DSi) and the N:P:Si ratio (Schelske and Stoermer 1971, Hecky and Kilham 1988, Conley et al. 1993, Turner et al. 1998). In general, if waters are rich in DSi then increased loading of the limiting nutrient (N or P) should promote growth of diatoms until DSi is depleted. Once DSi limits diatom production, nonsiliceous taxa may become dominant and the risk for cyanobacterial blooms is increased. This mechanism for generating nuisance and harmful blooms is proposed for both freshwater and marine ecosystems (Officer and Ryther 1980).

Significant nutrient loading to the northern Gulf of Mexico from the Mississippi-Atchafalaya River basin has been occurring for at least the last six decades (Turner and Rabalais 1991; Turner et al. 2007). The result of this nutrient loading is annual formation of a seasonal hypoxic zone below the pycnocline. River flow is a strong predictor of seasonal and annual N and P loading to the Gulf of Mexico and of the areal size and volume of the hypoxic zone (Scavia and Donnelly 2007; Turner et al. 2012; Obenour et al. 2013). The extent to which discharge affects the stoichiometry of nutrient loads in the Mississippi River is not fully resolved. Leong et al. (2014) reported that for freshwater delivered to the Gulf of Mexico by the Mississippi and Atchafalaya Rivers, N:Si ratios increased with discharge but the effect was not large: a doubling of discharge increased N:Si by 4%. Leong et al. (2014) did not directly examine P:Si in their analysis, but they did show that P had a more linear response to discharge than did Si (power law exponents of 1.07 and 1.13 for P and Si, respectively). Years with high discharge through the Mississippi and Atchafalaya Rivers should therefore have nutrient loads with lower P:Si ratios and reduced risk for blooms of cyanobacteria or other harmful groups.

*Correspondence: troyer@indiana.edu

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Additional Supporting Information may be found in the online version of this article.
Long-term changes in stoichiometry of the nutrient loads have resulted in changes in the phytoplankton community of the northern Gulf of Mexico (Turner et al. 1998). A decline in DSi concentrations since the mid-20th century has occurred in water entering the Gulf from the Mississippi-Atchafalaya River basin (hereafter, Mississippi basin), with concomitant increases in N:Si and P:Si ratios (Turner et al. 2007). A proposed cause for the decline in DSi in the Mississippi River is retention of biogenic silica in the sediment of eutrophic reservoirs throughout the Mississippi basin. An inverse relationship between dissolved Si concentrations in the Mississippi River and the mass of P fertilizer used in the U.S. provides support for this hypothesis (Turner and Rabalais 1991). Others argue that increased N:Si ratios are the result of N runoff from agriculture and that reservoirs play a minor role (Downing et al. 2016). In the latter case, regions of the Mississippi basin dominated by agriculture should disproportionately affect N:Si stoichiometry of nutrient loads received by the Gulf of Mexico. In either case, there is reason to expect that stoichiometry of the nutrient loads of the major tributaries to the Mississippi River have shifted toward conditions that favor cyanobacterial blooms when light is not limiting, such as in the coastal zone or in rivers with an open canopy. The Microcystis bloom that covered >1000 km of the Ohio River in October of 2015 (ORSANCO 2016) demonstrated that extensive cyanobacterial blooms are possible even in the generally turbid rivers of the Mississippi basin.

Billen and Garnier (2007) presented the indicator of coastal eutrophication potential (ICEP) as a tool for determining the stoichiometric imbalance in N:Si or P:Si and thus the potential for growth of nonsiliceous phytoplankton. Because harmful algal blooms are not typically the result of diatoms (but see Bargu et al. 2016), the ICEP has been viewed as an indicator of the potential for cyanobacterial blooms (Garnier et al. 2010a; Gilbert et al. 2017). The ICEP has units of kg C km\(^{-2}\) d\(^{-1}\) and represents the mass of carbon (C) that could be fixed by nonsiliceous phytoplankton given the amount of N or P available in excess of stoichiometric balance with Si, based on the Redfield ratio for diatoms of 106C:16 N:1P:20Si. The ICEP differs from simple nutrient ratios because it incorporates both loads and stoichiometry, and thus describes stoichiometric imbalances in absolute terms. The ICEP can be calculated on the basis of N:Si (hereafter, N-ICEP) if N is the expected limiting nutrient for algal growth, or on the basis of P:Si if P is limiting (hereafter, P-ICEP). In both cases, values >0 indicate excess N or P relative to DSi in the riverine load, with the value increasing in proportion to the stoichiometric imbalance. Values <0 indicate DSi in excess of N or P and a low probability of blooms of nonsiliceous algae.

The ICEP has been used to assess the risk for cyanobacterial blooms regionally and globally. Regional studies have centered on Europe and China, including analyses of interannual and intra-annual variability in the ICEP (Garnier et al. 2010b; Romero et al. 2013) and forecasts of future risk of cyanobacterial blooms using scenarios of changing N, P, and Si loading (Strokal and Kroeze 2013; Strokal et al. 2017). Regional or river-specific analyses have focused on rivers and time periods with reliable annual load estimates for N, P, and Si. Global-scale analyses have coupled the ICEP to the Global-NEWS model that predicts N, P, and Si yields across the Earth’s land surface (Seitzinger et al. 2010). This has allowed estimates of the ICEP for coastal zones at the mouths of unmonitored rivers (Garnier et al. 2010a). River basins with extensive human activity tend to have N- and P-ICEP values >1.0 kg C km\(^{-2}\) d\(^{-1}\), with measured values as high as 40–50 kg C km\(^{-2}\) d\(^{-1}\) for nutrient-rich European rivers (Garnier et al. 2010b, Romero et al. 2013). Garnier et al. (2010a) report a modeled N-ICEP for the freshwater inputs to the Gulf of Mexico in the range of 0–2 kg C km\(^{-2}\) d\(^{-1}\), but to date, the ICEP has not been calculated using measured nutrient loads for the Mississippi basin. Therefore, the purpose of this article is to examine spatial patterns and temporal trends in the N:Si stoichiometry of the Mississippi River and its major subbasins using the ICEP approach for the 1979–2015 water years.

The first objective of this article is to examine patterns in the ICEP that could inform our understanding of nutrient loss from the landscape of the Mississippi basin. Specifically, I seek to answer the following questions: (1) Over the period of record, do the N- or P-ICEP differ statistically from 0, thus indicating potential for cyanobacterial blooms? (2) Are the stoichiometric imbalances consistent across the major subbasins of the Mississippi River? (3) Does the intensively agricultural Upper Mississippi subbasin control N:Si stoichiometry of water received in the northern Gulf of Mexico, as suggested by Downing et al. (2016)?

The second objective is to examine temporal patterns in the ICEP at both interannual and intra-annual time scales. There is evidence of increasing P concentrations across all surface waters within the contiguous U.S. (Stoddard et al. 2016) and cyanobacterial blooms are a persistent problem throughout the Mississippi basin. There are no consistent temporal trends across the Mississippi basin in N concentrations or loads. Some rivers appear to have declining loads (Mclssac et al. 2016) while others have increasing or unchanging concentrations and loads (Sprague et al. 2013). Consequently, I expected P-ICEP values to have increased over the period of record, but expected no consistent interannual trends in the N-ICEP for the Mississippi River or its major subbasins. With regard to intra-annual (monthly) patterns, I expected the freshwater inputs to the Gulf of Mexico to most strongly favor nonsiliceous algae during late winter and early spring, when the bulk of the nutrient loss occurs in the agricultural regions of the Mississippi basin (e.g., Royer et al. 2006). The final objective is to determine if the N- or P-ICEP were correlated with mean annual discharge in the Mississippi River or its subbasins during the period of record.

**Methods**

**Description of study basins**

This analysis focused on the Mississippi River basin and four of its major subbasins. The subbasins included the Upper Mississippi, the Ohio-Tennessee, the Missouri, and the Arkansas-Red
Combined, these four subbasins comprise 94.3% of the land area contributing runoff to the northern Gulf of Mexico through the Mississippi and Atchafalaya Rivers (the Atchafalaya is a distributary of the Mississippi that discharges to the Gulf of Mexico northwest of the Mississippi River delta). The subbasins span more than a threefold range in mean daily discharge and nearly an order of magnitude in runoff, from 6.5 cm yr\(^{-1}\) in the Missouri basin to 50.2 cm yr\(^{-1}\) in the Ohio-Tennessee basin (Table 1). Land use and other characteristics of the study basins have been described in detail previously (Goolsby et al. 1999; US EPA 2007). In brief, the Upper Mississippi and Ohio-Tennessee basins contribute >80% of annual nitrate load delivered to the Gulf of Mexico from the Mississippi and Atchafalaya Rivers, and >60% of the total phosphorus load (US EPA 2007). During wet years, small watersheds dominated by row-crop agriculture in the Upper Mississippi and Ohio-Tennessee basins can have annual nitrate-N and total phosphorus yields >50 kg ha\(^{-1}\) and 1 kg ha\(^{-1}\), respectively (Royer et al. 2006). Other portions of the Mississippi basin are comparatively minor sources of N and P to the Gulf of Mexico, although nutrient enrichment of surface waters is pervasive in much of the basin.

Data sources and ICEP calculation

Mean annual or monthly discharge and loads of NO\(_3^-\) + NO\(_2^-\), NH\(_4^+\), total P, and SiO\(_2\) in the study basins for the 1979–2015 water years, or portions thereof (Table 1), were obtained from the U.S. Geological Survey (USGS; https://nrtwq.usgs.gov/mississippi_loads/#/). The USGS determined the annual loads using adjusted maximum likelihood estimation in LOADEST, a model-fitting program that uses continuous discharge records and periodic nutrient concentrations to estimate nutrient loads on monthly or annual time steps (Aulenbach et al. 2007; Lee et al. 2017). For calculation of the ICEP, NO\(_3^-\) + NO\(_2^-\) and NH\(_4^+\) loads (as N) were summed to dissolved inorganic nitrogen and dissolved SiO\(_2\) was expressed as the mass of Si. I opted to use inorganic N because it represents the majority of the total N in the Mississippi River and its major tributaries (Goolsby et al. 1999) and previous investigations on N:Si stoichiometry in the Mississippi River (Turner et al. 1998, 2007) and the ICEP (Garnier et al. 2010\(a\)) have used inorganic N rather than total N. The one exception to this was for the monthly estimates of N-ICEP, in which only NO\(_3^-\) + NO\(_2^-\) was used because of limited availability of monthly NH\(_4^+\) loads. ICEP was calculated as presented by Billen and Garnier (2007) and based on an elemental ratio in diatoms of 106C:16N:1P:20Si:

\[
\text{N-ICEP (kg C km}^{-2} \text{d}^{-1}) = \frac{[\text{N yield}/(14 \times 16) - \text{DSi yield}/(28 \times 20)]}{12 \times 106}
\]

(1)

\[
\text{P-ICEP (kg C km}^{-2} \text{d}^{-1}) = \frac{[\text{P yield}/31 - \text{DSi yield}/(28 \times 20)]}{12 \times 106}
\]

(2)

where yields are expressed as kg C km\(^{-2}\) d\(^{-1}\) for each nutrient. Daily yields were calculated from annual or monthly loads and basin drainage areas (Table 1). Equation 1 expresses the imbalance between inorganic N and DSi in terms of the amount of C that could be fixed with the excess N, assuming N as the limiting nutrient for algal growth (Billen and Garnier 2007). Equation 2 is the corresponding calculation for P-limited conditions.

![Fig. 1. Map of the Mississippi-Atchafalaya River basin (bold outline) and the four major subbasins used in the analysis. The USGS gaging stations used for calculating nutrient loads are indicated (see Aulenbach et al. 2007 for details).](image-url)
Regardless of the limiting nutrient, the ICEP provides a quantitative expression of N, P, and Si stoichiometric imbalance in annual or monthly nutrient loads, where the balanced condition is that which favors growth of diatoms over nonsiliceous algae.

The relative requirement for N, P, and Si by diatoms is a key component of the ICEP and strongly influences whether a given set of N, P, and Si loads results in positive or negative values of the ICEP. Si depletion is more likely to occur, or to occur more quickly, in a diatom community with high Si demand relative to N or P. The ICEP uses 16N:20Si and 1P:20Si, but it is important to recognize that there is variation among diatom species in Si requirements and that a given diatom community could deviate from the ratios used in the ICEP. For example, Brzezinski (1985) grew cultures of 27 marine and coastal diatom species under controlled conditions and reported an average N:Si of 16:15. Freshwater diatoms have a greater silicon content than marine diatoms (e.g., Conley and Kilham 1989), and on this basis, Dupas et al. (2015) modified the ICEP for freshwater by using a ratio of 16N:1P:40Si. In this article, I focus mainly on the original form of the ICEP because it is most relevant to the Gulf of Mexico. However, I also calculated the indicator of freshwater eutrophication potential (IFEP) as described by Dupas et al. (2015) for the freshwater subbasins to explore how a greater Si demand affects the risk for cyanobacterial blooms.

### Statistical analysis

A one-sample Wilcoxon sign rank test was used to determine if the median of the distribution of monthly or annual N- and P-ICEP values was greater than zero for each site. Simple linear regression was used to examine the influence of the Upper Mississippi subbasin on N-ICEP of water delivered to the Gulf of Mexico by the Mississippi and Atchafalaya Rivers. The nutrient loads of the Mississippi and Atchafalaya Rivers are not wholly independent of the Upper Mississippi subbasin; therefore, the resulting model should be viewed as predictive rather than explanatory. To determine if the N- and P-ICEP had changed over time, data from the 1980–1994 period were compared to the 2001–2015 period using the Mann–Whitney test. The 1980–1994 period corresponds approximately to the baseline period used for setting nutrient load reduction goals for the Gulf of Mexico (US EPA 2007); data from the Upper Mississippi River subbasin were available only for 1980–1986. Complete records were available for the second 15-yr period for all but the Arkansas-Red subbasin, which was limited to 2004–2015. Last, a Pearson product-moment correlation analysis was used to examine relationships between the N- or P-ICEP and mean annual discharge across the study basins. For both regressions and correlations, relationships were considered significant at α = 0.05. Statistical analyses were conducted in Minitab v17 (Minitab, State College, Pennsylvania) or SigmaStat v4 (Systat Software, San Jose, California).

### Results

Over the period of record, the median P-ICEP was statistically greater than 0 (Wilcoxon test, p < 0.001) for all but the Arkansas-Red subbasin, and the median N-ICEP was > 0 for all but Arkansas-Red and Missouri subbasins (Fig. 2). The Upper Mississippi subbasin had the highest values, with a median for the N- and P-ICEP of 6.2 kg C km\(^{-2}\) d\(^{-1}\) and 3.2 kg C km\(^{-2}\) d\(^{-1}\), respectively. In fact, no year in the period of record for the Upper Mississippi subbasin had an N-ICEP ≤ 0, indicating annual dissolved inorganic N loads were always in excess of stoichiometric balance with annual DSi loads. Conversely, the N-ICEP for the Arkansas-Red subbasin was never > 0, indicating an excess of DSi relative to annual inorganic N loads in every year.

Plotting the N- and P-ICEP against each other shows clearly the high N and P loading, relative to DSi, in the Upper Mississippi and Ohio-Tennessee subbasins, with nearly all years positive for both the N- and P-ICEP (Fig. 3). Only three water years for the Upper Mississippi subbasin (1983, 1984, and 2015) had negative P-ICEP values, and each of those years had annual P loads lower than the average for the period of record.
and higher than average DSi loads. Figure 3 also indicates that P loading in the Missouri subbasin is imbalanced to a greater extent than is N loading, with all years near or below 0 for the N-ICEP while 33 of 36 yr had P-ICEP > 0.

The agriculturally dominated Upper Mississippi subbasin explained 56% of the annual variation in the N-ICEP of the combined Mississippi and Atchafalaya Rivers (Fig. 4). The y-intercept of the regression model was negative, meaning that if the Upper Mississippi subbasin contributed stoichiometrically balanced loads of inorganic N and DSi (i.e., N-ICEP = 0), the input to the Gulf of Mexico from the Mississippi basin would contain DSi in excess of inorganic N relative to the stoichiometric requirements of diatoms.

The N-ICEP in the Ohio-Tennessee subbasin during 2001–2015 was significantly lower than in the 1980–1994 period, with median values of 3.0 kg C km$^{-2}$ d$^{-1}$ and 1.7 kg C km$^{-2}$ d$^{-1}$ for the earlier and later periods, respectively (Fig. 5). No other subbasin had a significant change in the N-ICEP, nor did the Mississippi basin as a whole. Conversely, the P-ICEP increased significantly in all of the study basins except the Arkansas-Red (Fig. 5). The median P-ICEP increased by 22-fold in the Ohio-Tennessee subbasin, from 0.15 to 3.3 kg C km$^{-2}$ d$^{-1}$. In the Missouri and Upper Mississippi subbasins, the median P-ICEP increased by sixfold and ninefold, respectively. For the inputs to the Gulf of Mexico from the Mississippi and Atchafalaya Rivers, the median P-ICEP more than doubled, from 0.71 to 1.7 kg C km$^{-2}$ d$^{-1}$. To the extent that nutrient loads from the subbasins reflect surface waters in general, the P-ICEP results indicate stoichiometric changes over the last four decades that favor growth of cyanobacteria throughout most of the Mississippi basin.

There was a clear intra-annual pattern in the N-ICEP for water entering the Gulf of Mexico through the Mississippi and Atchafalaya Rivers, but a much less distinct pattern for the P-ICEP (Fig. 6). Over the 1980–2015 water years, the median monthly P-ICEP was statistically > 0 for each month (Wilcoxon test, $p < 0.01$), indicating a consistent excess of P relative to DSi. For the N-ICEP, March through August had median values statistically > 0, although the median value for August was only 0.2 kg C km$^{-2}$ d$^{-1}$ (Fig. 6). By late summer, nitrate and DSi were roughly in balance and the N-ICEP values were generally negative for the remainder of most years.

There was a weak but significant correlation between the P-ICEP for the inputs to the Gulf of Mexico and mean annual
discharge of the combined Mississippi and Atchafalaya Rivers (Pearson $r = -0.38$, $p = 0.024$, Fig. 7). The inverse relationship indicates that $P$ and $DSi$ loads in years with high river flow are less imbalanced than are loads during years with low or moderate river flow. No correlation existed between the N-ICEP and mean annual discharge for the combined Mississippi and Atchafalaya Rivers. Among the subbasins, correlations between N- or P-ICEP and discharge were generally nonsignificant or weak (Supporting Information Table S1), with the exception of the N-ICEP for the Arkansas-Red which was strongly and inversely correlated to mean annual discharge (Pearson $r = -0.83$, $p < 0.001$, Supporting Information Fig. S1).

Table 2 presents the mean, minimum, and maximum values for N-IFEP and P-IFEP for each subbasin. A linear regression of N-IFEP against N-ICEP had an intercept of 1.86 and a slope of 1.26 ($p < 0.001$, $R^2 = 0.91$). The regression of P-IFEP against P-ICEP had an intercept of 2.83 and slope of 1.34 ($p < 0.001$, $R^2 = 0.66$) (see Supporting Information Fig. S2). The higher silica demand used in the IFEP results in a greater rate of increase in stoichiometric imbalance (i.e., regression slopes > 1.0) across the range of observed nutrient loads in the subbasins. However, the overall patterns within and among subbasins were not different between the IFEP and the ICEP.

![Fig. 4. Least squares regression of the N-ICEP for the Mississippi-Atchafalaya input to the Gulf of Mexico against the N-ICEP of the Upper Mississippi subbasin. The dashed lines indicate the 95% confidence limits. $n = 27$ and includes the 1980–1986 and 1996–2015 water years.](image)

![Fig. 5. Box plots of the P-ICEP (top row) and N-ICEP (bottom row) during two 15-yr time periods, the 1980–1994 and 2001–2015 water years (or portions thereof) for each of the study basins. Results of Mann–Whitney tests are shown for those basins with significant differences between the two time periods. Box plots as described in Fig. 2.](image)
Discussion

The N-ICEP values reported here using measured nutrient loads for the Mississippi basin agree with those estimated by Garnier et al. (2010a) of 0–2 kg C m\(^{-2}\) d\(^{-1}\). The nutrient loads from the Mississippi basin drive formation of a seasonal hypoxic zone in the northern Gulf of Mexico that is among the largest in the world, often exceeding 10,000 km\(^2\) and occasionally exceeding 20,000 km\(^2\) (Matli et al. 2018). However, the stoichiometric imbalance based on the N-ICEP is an order of magnitude less than that reported for the Seine River and other European rivers draining landscapes with high population densities (Billen and Garnier 2007; Garnier et al. 2010b). The spatial extent of the Gulf hypoxic zone is determined largely by the mass of N delivered by the Mississippi River with stoichiometry of the nutrient loads likely playing a secondary role (Turner et al. 2012; Scavia et al. 2017). The ICEP is based on the relative yields (kg km\(^{-2}\)) of N, P, and Si rather than total mass, so it is not expected that it would predict the size of the hypoxic zone. Rather, the ICEP reveals the degree to which nutrient loads are imbalanced with respect to the N, P, and Si requirements of diatoms and, by extension, the likelihood of blooms of non-siliceous groups, particularly cyanobacteria.

The ICEP and phytoplankton in the northern Gulf of Mexico

On an annual basis, the N-ICEP for the freshwater inputs from the Mississippi and Atchafalaya Rivers suggests that there
is potential for blooms of nonsiliceous groups in the northern Gulf of Mexico, but harmful cyanobacteria have thus far not dominated phytoplankton biomass (Dortch et al. 2001; Chakraborty and Lohrenz 2015). Stoichiometry of nutrient inputs is only one factor that could influence bloom formation and an N-ICEP value > 0 does not mean harmful cyanobacterial blooms are inevitable. A recent review highlighted the interacting roles of turbidity, temperature, salinity, nutrient availability, and stratification in structuring phytoplankton communities in the northern Gulf of Mexico (Bargu et al. 2019). Despite the potential for silica limitation (Dortch and Whitledge 1992), diatoms are frequently reported as a dominant group within nearshore phytoplankton communities, along with chlorophytes and cryptophytes (e.g., Schaeffer et al. 2012; Chakraborty and Lohrenz 2015). Periods of high discharge from Mississippi and Atchafalaya Rivers, which occur mainly in spring, create conditions of relatively high turbidity and nutrients but low salinity, and these conditions appear to favor diatoms, chlorophytes, and cryptophytes, rather than cyanobacteria which prefer conditions that occur later in summer (Bargu et al. 2019). The monthly patterns in the N-ICEP (Fig. 6) show that freshwater inputs favor nonsiliceous groups in spring and early summer, but during this time, temperature and other conditions are not conducive for cyanobacterial blooms. By mid to late summer, when temperature and other factors are favorable for cyanobacteria, the stoichiometry of the freshwater inputs favors diatoms over nonsiliceous groups.

Spatial patterns among the subbasins of the Mississippi River

The predominant land use varies among the major subbasins of the Mississippi River and this was clearly reflected in the N- and P-ICEP values. The intensively farmed and fertilized Upper Mississippi subbasin is the largest source of N to the Gulf of Mexico (US EPA 2007). Based on the N-ICEP, the Upper Mississippi is also the driver of stoichiometric imbalance in nutrient loads delivered to the Gulf. In terms of land area, the Upper Mississippi is the smallest of the subbasins examined, representing only 15% of the total Mississippi-Atchafalaya River basin (Table 1). But the high N and P yields, relative to DSi yields, make this subbasin the key determinant not only of the mass of annual N and P loads but also the stoichiometry of the annual loads. The N-ICEP results thus support the argument by Downing et al. (2016) that N runoff from agriculture, as opposed to Si retention in reservoirs, is the primary driver of N:Si stoichiometry in surface waters of the Upper Mississippi subbasin.

Human activities in general, and agriculture in particular, tend to increase both N and P loading to surface waters. In a global analysis, Garnier et al. (2010a) reported 83% of rivers with positive N-ICEP values also had positive P-ICEP values. This was the case for the Upper Mississippi and Ohio-Tennessee subbasins for which nearly all years fall in the upper right quadrant of Fig. 3, indicating positive values of both the N- and P-ICEP. However, the Missouri subbasin rarely had positive values of N-ICEP while consistently having positive values of P-ICEP, and the Arkansas-Red subbasin was generally negative for both the N- and P-ICEP. There are two significant implications from the patterns among subbasins. First, in terms of the ICEP, the freshwater input from the Mississippi and Atchafalaya Rivers is most similar to the Upper Mississippi and Ohio-Tennessee subbasins (Fig. 3), further highlighting the effect of these agriculturally intensive subbasins on nutrient conditions in the northern Gulf of Mexico. Second, assuming P-ICEP calculated at the monitoring stations (Fig. 1) generally reflects conditions in surface waters of each subbasin, the risk of cyanobacterial blooms appears low in the Arkansas-Red but high in the other subbasins. The extent to which this assumption is valid is unknown, and undoubtedly local cyanobacterial blooms occur within the Arkansas-Red subbasin despite the low P-ICEP values at the mouths of the rivers. The potential for cyanobacterial blooms within the subbasins is further emphasized by the IFEP, which uses a silicon content for diatoms that is twice that used in the ICEP. Because of the greater silicon content of freshwater diatoms, a given stoichiometric imbalance in nutrient loads generates a greater potential for cyanobacterial blooms in the inland waters of the Mississippi basin than in the northern Gulf of Mexico.

Non N₂-fixing cyanobacteria can form toxin-producing blooms and strong arguments exist for dual N and P control to reduce eutrophication and harmful algal blooms (e.g., Paerl 2009; Scott and McCarthy 2010). The ICEP results presented here show that for the Upper Mississippi and Ohio-Tennessee subbasins both N and P loading are far in excess of DSi, relative to diatom requirements. Reduced N and P loading in those subbasins is likely to generate the largest effect on the N- or P-ICEP of the inputs to the Gulf, and any reduction in N or P loading within a subbasin is likely to have positive effects locally. However, Dortch et al. (2001) cautioned that small reductions in P loading to inland waters of the Mississippi basin could exacerbate Gulf hypoxia by increasing DSi delivery to the Gulf during periods when diatom production is silica-limited. This scenario arises from the fact that much of the organic matter fueling the respiration in the hypoxic zone derives from diatom production in the surface waters of the Gulf (Turner and Rabalais 1994). Reduced P loading in the Mississippi basin could potentially reduce DSi uptake by diatoms in the inland waters of the basin, allowing more DSi to reach the Gulf of Mexico and further stimulate production by diatoms. At present, P loading in the Mississippi basin appears to be increasing (Stoddard et al. 2016, Supporting Information Fig. S3) and therefore, the situation described by Dortch et al. (2001) is not imminent. Nonetheless, it points out a reason to consider stoichiometric responses to N and P management, something that is not commonly done particularly in reference to distant downstream waters.

Temporal patterns in the ICEP

Across the Mississippi basin, the N-ICEP has mostly remained unchanged from the early portion of the period of record (1980–1994) to the later portion (2001–2015), despite significant
efforts (and expenditures) during the last decade to reduce N loading from cropland (e.g., USDA 2012). The N-ICEP declined in the Ohio-Tennessee subbasin but remained positive with a median for the 2001–2015 period of about 2 kg C km$^{-2}$ d$^{-1}$ (Fig. 5). The significant increase in the P-ICEP over the period of record signals growing risk for cyanobacterial blooms across most of the Mississippi basin. There currently is no standardized and quantitative surveillance program for harmful algal blooms in the U.S. (Brooks et al. 2016) which precludes empirically linking the changes in the P-ICEP to increased severity or frequency of cyanobacterial blooms within the study basins. Nonetheless, the P-ICEP results suggest more favorable conditions for cyanobacteria, which is consistent with anecdotal trends of increasing frequency and severity of harmful blooms with in the Mississippi basin and elsewhere (Brooks et al. 2016). Loads of DSI have not changed appreciably over the period of record (Supporting Information Fig. S4) confirming that the changes in the P-ICEP are the result of increased P loading, such as that documented by Stoddard et al. (2016). The ICEP has the potential to serve as an integrative biogeochemical monitoring tool that can be applied across spatial and temporal scales. Where appropriate data are available, the ICEP could aid in identifying outcomes from nutrient management or other agricultural conservation practices which are challenging to detect in records of nutrient loads, particularly at large spatial scales (García et al. 2016).

**Discharge and the stoichiometry of nutrient loads**

The positive relationship between discharge and N loads in the Mississippi River and its tributaries is well established (e.g., Donner et al. 2002; Raymond et al. 2012). The lack of correlation between the N-ICEP and river flow (Fig. 7B) thus suggests that the stoichiometry of nutrient loads is less sensitive to hydrological condition than is the mass of the nutrient loads. Among the subbasins, the only strong correlation was an inverse relationship between the N-ICEP and discharge for the Arkansas-Red (Supporting Information Fig. S1). Leong et al. (2014) reported that, for both the Arkansas River and the Red River, DSI had a stronger nonlinear response to increasing discharge than did N. If DSI is more strongly affected by discharge than is N, then it would generate the inverse relationship between the N-ICEP and discharge shown in Supporting Information Fig. S1. The general correspondence in results between this study and that of Leong et al. (2014) is due in part to the fact that both use the same nutrient load data from the U.S. Geological Survey. But the analytical approaches are quite different, which provides strengthened confidence in the conclusion that mass of nutrient loads and stoichiometry of nutrient loads respond differently to hydrological conditions.

**References**

Aulenbach, B. T., H. T. Buxton, W. A. Battaglin, and R. H. Coupe. 2007. Streamflow and nutrient fluxes of the Mississippi-Atchafalaya River Basin and subbasins for the period of record through 2005. U.S. Geological Survey Open-File Report 2007-1080. Available from https://toxics.usgs.gov/pubs/of-2007-1080/

Bargu, S., M. M. Baustian, N. N. Rabalais, R. Del Rio, B. Von Korff, and R. E. Turner. 2016. Influence of the Mississippi River on *Pseudo-nitzschia* spp. abundance and toxicity in Louisiana coastal waters. Estuaries Coast. 39: 1345–1356. doi:10.1007/s12237-016-0088-y

Bargu, S., D. Justic, J. R. White, R. Lane, J. Day, J. Paerl, and R. Raynie. 2019. Mississippi River diversions and phytoplankton dynamics in the deltaic Gulf of Mexico estuaries: A review. Estuar. Coast. Shelf Sci. 221: 39–52. doi:10.1016/j.ecss.2019.02.020

Billen, G., and J. Garnier. 2007. River basin nutrient delivery to the coastal sea: Assessing its potential to sustain new production of non-siliceous algae. Mar. Chem. 106: 148–160. doi:10.1016/j.marchem.2006.12.017

Brooks, B. W., and others. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? Environ. Toxicol. Chem 35: 6–13. doi:10.1002/etc.3220

Brzezinski, M. A. 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. J. Phycol. 21: 347–357.

Chakraborty, S., and E. S. Lohrenz. 2015. Phytoplankton community structure in the river-influenced continental margin of the northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 521: 31–47. doi:10.3354/meps11107

Conley, D. J., and S. S. Kilham. 1989. Differences in silica content between marine and freshwater diatoms. Limnol. Oceanogr. 34: 205–213. doi:10.4319/lo.1989.34.1.0205

Conley, D. J., C. L. Schelske, and E. F. Stoermer. 1993. Modifi- cation of the biogeochemical cycle of silica with eutrophication. Mar. Ecol. Prog. Ser. 101: 179–192. doi:10.3354/meps101179

Donner, S. D., M. T. Coe, J. D. Lenters, T. E. Twine, and J. A. Foley. 2002. Modeling the impact of hydrological changes on nitrate transport in the Mississippi River Basin from 1955 to 1994. Global Biogeochem. Cycles 16: 16-1-16-19. doi:10.1029/2001GB001396

Dortch, Q., and T. E. Whitledge. 1992. Does nitrogen or silicon limit phytoplankton production in the Mississippi River plume and nearby regions? Cont. Shelf Res. 12: 1293–1309. doi:10.1016/0278-4343(92)90065-R

Dortch, Q., N. N. Rabalais, R. E. Turner, and N. A. Qureshi. 2001. Impacts of changing Si/N ratios and phytoplankton species composition, p. 37–48. In N. N. Rabalais and R. E. Turner [eds.], Coastal hypoxia consequences for living resources and ecosystems. American Geophysical Union.

Downing, J. A., C. T. Cherrier, and R. W. Fulweiler. 2016. Low ratios of silica to dissolved nitrogen supplied to rivers arise from agriculture not reservoirs. Ecol. Lett. 19: 1414–1418. doi:10.1111/ele.12689

Dupas, R., M. Delmas, J. M. Dorioz, J. Garnier, F. Moatar, and C. Gascoel-Odoux. 2015. Assessing the impact of
agricultural pressures on N and P loads and eutrophication risk. Ecol. Indic. 48: 396–407. doi:10.1016/j.ecolind.2014.08.007

García, A. M., R. B. Alexander, J. G. Arnold, L. Norfleet, M. J. White, D. M. Robertson, and G. Schwarz. 2016. Regional effects of agricultural conservation practices on nutrient transport in the upper Mississippi River basin. Environ. Sci. Technol. 50: 6991–7000. doi:10.1021/acs.est.5b03543

Garnier, J., A. Beusen, V. Thieu, G. Billen, and L. Bouwman. 2010a. N:P:Si nutrient export ratios and ecological consequences in coastal seas evaluated by the ICEP approach. Global Biogeochem. Cycles 24: GB0A05. doi:10.1029/2009GB003583

Garnier, J., G. Billen, J. Némery, and M. Sebilo. 2010b. Transformations of nutrients (N, P, Si) in the turbidity maximum zone of the Seine estuary and export to the sea. Estuar. Coast. Shelf Sci. 90: 129–141. doi:10.1016/j.ecss.2010.07.012

Glibert, P. M., A. H. W. Beusen, J. A. Harrison, H. H. Dürr, A. F. Bouwman, and G. G. Laruelle. 2017. Changing land-, sea-, and airscapes: Sources of nutrient pollution affecting habitat suitability for harmful algae, p. 53–76. In P. M. Glibert, E. Berdalet, M. A. Burford, G. C. Pitcher, and M. Zhou [eds.], Global ecology and oceanography of harmful algal blooms. Springer.

Goolsby, D. A., W. A. Battaglin, G. B. Lawrence, R. S. Artz, B. T. Aulenbach, R. P. Hooper, D. F. Keeney, and G. J. Stensland. 1999. Flux and sources of nutrients in the Mississippi-Atchafalaya River basin: Topic 3 report for the integrated assessment of hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program Decision Analysis Series No. 17. NOAA Coastal Ocean Program.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. 33: 796–822. doi:10.4319/lo.1988.33.4part2.0796

Lee, C. J., J. C. Murphy, C. G. Crawford, and J. R. Deacon. 2017. Methods for computing water-quality loads at sites in the U.S. Geological Survey National Water Quality Network. U.S. Geological Survey Open-File Report 2017–1120; [accessed 2019 June 28]. Available from https://doi.org/10.3133/ofr20171120

Leong, D. N. S., S. D. Donner, M. A. Hassan, R. Gabor, and J. D. Drummond. 2014. Sensitivity of stoichiometric ratios in the Mississippi River to hydrologic variability. J. Geophys. Res. Biogeosci. 119: 1049–1062. doi:10.1002/2013jg002585

Matli, V. R. R., S. Fang, J. Guinness, N. N. Rabalais, J. K. Craig, and D. R. Obenour. 2018. Space–time geostatistical assessment of hypoxia in the northern Gulf of Mexico. Environ. Sci. Technol. 52: 12484–12493. doi:10.1021/acs.est.8b03474

McIissac, G. F., M. B. David, and G. Z. Gertner. 2016. Illinois River nitrate-nitrogen concentrations and loads: Long-term variation and association with watershed nitrogen inputs. J. Environ. Qual. 45: 1268–1275. doi:10.2134/jeq2015.10.0531

Obenour, D. R., D. Scavia, N. N. Rabalais, R. E. Turner, and A. M. Michalak. 2013. Retrospective analysis of midsummer hypoxic area and volume in the northern Gulf of Mexico, 1985-2011. Environ. Sci. Technol. 47: 9808–9815. doi:10.1021/es400983g

Officer, C. B., and J. H. Ryther. 1980. The possible importance of silicon in marine eutrophication. Mar. Ecol. Prog. Ser. 3: 83–91. doi:10.3354/meps003083

ORSANCO. 2016. Annual report 2016. Ohio River Valley Sanitary Commission (ORSANCO); [accessed 2019 May 29]. Available from http://www.orsanco.org/wp-content/uploads/2016/07/2016_Annual_report_web.pdf

Paerl, H. W. 2009. Controlling eutrophication along the freshwater-marine continuum: Dual nutrient (N and P) reductions are essential. Estuaries Coast. 32: 593–601. doi:10.1007/s12237-009-9158-8

Raymond, P. A., M. B. David, and J. E. Saiers. 2012. The impact of fertilization and hydrology on nitrate fluxes from Mississippi watersheds. Curr. Opin. Environ. Sustain. 4: 212–218. doi:10.1016/j.cosust.2012.04.001

Romero, E., J. Garnier, L. Lassaletta, G. Billen, R. Le Gendre, P. Riou, and P. Cugier. 2013. Large-scale patterns of river inputs in southwestern Europe: Seasonal and interannual variations and potential eutrophication effects at the coastal zone. Biogeochemistry 113: 481–505. doi:10.1007/s10533-012-9778-0

Royer, T. V., M. B. David, and L. E. Gentry. 2006. Timing of riverine export of nitrate and phosphorus from agricultural watersheds in Illinois: Implications for reducing nutrients loading to the Mississippi River. Environ. Sci. Technol. 40: 4126–4131. doi:10.1021/es052573n

Scavia, D., and K. A. Donnelly. 2007. Reassessing hypoxia forecasts for the Gulf of Mexico. Environ. Sci. Technol. 41: 8111–8117. doi:10.1021/es0714235

Scavia, D., I. Bertani, D. R. Obenour, R. E. Turner, D. R. Forrest, and A. Katin. 2017. Ensemble modeling informs hypoxia management in the northern Gulf of Mexico. Proc. Natl. Acad. Sci. USA 114: 8823–8828. doi:10.1073/pnas.1705293114

Schaeffer, B. A., J. C. Kurtz, and M. K. Hein. 2012. Phytoplankton community composition in the nearshore coastal waters of Louisiana. Mar. Pollut. Bull. 64: 1705–1712. doi:10.1016/j.marpolbul.2012.03.017

Schelske, C. L., and E. F. Stoermer. 1971. Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. Science 173: 423–424. doi:10.1126/science.173.3995.423

Scott, J. T., and M. J. McCarthy. 2010. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnol. Oceanogr. 55: 1265–1270. doi:10.4319/lo.2010.55.3.1265

Seitzinger, S. P., and others. 2010. Global river nutrient export: A scenario analysis of past and future trends. Global Biogeochem. Cycles 24: GB0A08. doi:10.1029/2009GB003587
Sprague, L. A., J. C. Murphy, and R. M. Hirsch. 2013. Nitrate in the Mississippi River and its tributaries, 1980–2010: An update. United States Geological Survey Scientific Investigations Report 2013–5169. [accessed 2019 June 28]. Available from http://pubs.usgs.gov/sir/2013/5169

Stoddard, J. L., J. Van Sickle, A. T. Herlihy, J. Brahney, S. Paulsen, D. V. Peck, R. Mitchell, and A. I. Pollard. 2016. Continental-scale increase in lake and stream phosphorus: Are oligotrophic systems disappearing in the United States? Environ. Sci. Technol. 50: 3409–3415. doi:10.1021/acs.est.5b05950

Strokal, M., and C. Kroeze. 2013. Nitrogen and phosphorus inputs to the Black Sea in 1970-2050. Reg. Environ. Change 13: 179–192. doi:10.1007/s10113-012-0328-z

Strokal, M., C. Kroeze, M. Wang, and L. Ma. 2017. Reducing future river export of nutrients to coastal waters of China in optimistic scenarios. Sci. Total Environ. 579: 517–528. doi:10.1016/j.scitotenv.2016.11.065

Turner, R. E., and N. N. Rabalais. 1991. Changes in Mississippi River water quality this century: Implications for coastal food webs. BioScience 41: 140–147. doi:10.2307/1311453

Turner, R. E., and N. N. Rabalais. 1994. Coastal eutrophication near the Mississippi River delta. Nature 368: 619–621. doi:10.1038/368619a0

Turner, R. E., N. Qureshi, N. N. Rabalais, Q. Dortch, D. Justic, R. F. Shaw, and J. Cope. 1998. Fluctuating silicate:nitrate ratios and coastal plankton food webs. Proc. Natl. Acad. Sci. USA 95: 13048–13051. doi:10.1073/pnas.95.22.13048

Turner, R. E., N. N. Rabalais, R. B. Alexander, G. McIsaac, and R. W. Howarth. 2007. Characterization of nutrient, organic carbon, and sediment loads and concentrations from the Mississippi River into the northern Gulf of Mexico. Estuaries Coast. 30: 773–790. doi:10.1007/BF02841333

US EPA. 2007. Hypoxia in the northern Gulf of Mexico. An update by the EPA Science Advisory Board. EPA-SAB-08-003. U.S. Environmental Protection Agency. [accessed 2019 June 28]. Available from https://yosemite.epa.gov/sab/sabproduct.nsf/C3D2F27094E03F90852573B800601D93/$File/EPA-SAB-08-003complete.unsigned.pdf

USDA. 2012. Assessment of the effects of conservation practices on cultivated cropland in the Upper Mississippi River Basin. Conservation effects assessment project final report. United States Department of Agriculture, Natural Resources Conservation Service. [accessed 2019 June 28]. Available from https://www.nrcs.usda.gov/Internet/FSE/Documents/stellprdb1042093.pdf

Acknowledgments
I thank Helen Scales for constructing Fig. 1 and Dr. Adam Ward for helpful comments on a draft of this paper. Suggestions from two anonymous reviewers substantively improved the paper.

Conflict of Interest
None declared.