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RESEARCH ARTICLE

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Environmental drivers of cyanobacterial abundance and cyanotoxin production in backwaters of the Upper Mississippi River

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Abstract

High densities of cyanobacteria in aquatic ecosystems can cause impacts to ecosystem services because they serve as a poor-quality food resource, produce toxins and can indirectly cause a variety of other negative impacts to water quality. There are many hypotheses about the potential environmental drivers of variation in cyanobacterial abundance and toxicity, but these hypotheses have rarely been considered in combination and rarely been examined in large river ecosystems. Here, we use monthly data from backwater habitats of the Upper Mississippi River (UMR) to evaluate associations between environmental conditions and cyanobacterial abundance and toxicity (microcystin and anatoxin-a) that would be expected based on several hypotheses. Backwaters in the Mississippi River vary in flushing rate, temperature, turbidity, nutrient availability, water depth, and vegetative cover. We find support for hypotheses that suggest physical conditions in backwaters (flushing rate, temperature, turbidity, rooted vegetation cover, and water depth) and nutrient availability influence cyanobacterial abundance and toxicity. We then used structural equation modeling to incorporate several hypotheses into a causal modeling framework, which indicated that backwater connectivity (flushing) strongly influences cyanobacterial abundance via the regulation of water temperature, and that nutrient availability strongly influences the presence of microcystin concentrations above our detection limit. Our data suggest that management of backwater connectivity could influence cyanobacterial abundance and toxicity in UMR backwaters. Reconnecting backwaters (via alteration of levees) could serve as a local adaptation to minimize the effects of climate change and excessive nutrient loading.

KEYWORDS
anatoxin-a, backwaters, connectivity, cyanobacteria, flushing, microcystin, Mississippi River, temperature

1 | INTRODUCTION

Aquatic ecosystems provide a variety of ecosystem services, including those related to recreation, drinking water, fisheries, and wildlife, to surrounding communities. These ecosystem services can be negatively impacted by the occurrence of harmful cyanobacterial blooms. Cyanobacterial blooms are harmful for a variety of reasons. Cyanobacteria produce compounds that are toxic to many consumers, including humans, and these compounds can reach high concentrations during blooms (Metcalf & Codd, 2012). Cyanobacteria are an intrinsically poor-quality food resource because they lack many of the fatty acids that appear to be essential for the growth of consumers (Brett & Müller-Navarra, 1997; Müller-Navarra et al., 2004). Dense blooms of cyanobacteria can create hypoxic zones by blocking light penetration to benthic primary producers, night-time respiration, and the decomposition of their bodies when they die (Watson et al., 2016; Zilius et al., 2014). Cyanobacteria blooms can also cause decreased water clarity, reduced macrophytes, and increase fish kills (Paerl & Otten, 2013). As a result, there is a widespread interest in identifying environmental conditions that drive variation in cyanobacterial abundance and toxicity.

The literature on environmental controls over cyanobacterial abundance and toxicity is extensive, with many competing and overlapping hypotheses (see overviews in Oliver, Hamilton, Brookes, & Ganf, 2012; Omidi, Esterhuizen-Londt, & Pflugmacher, 2018; Table 1). These include hypotheses related to the unique physiological abilities of cyanobacteria such as their ability to use atmospheric nitrogen

TABLE 1 Hypothesized environmental drivers of cyanobacterial biovolume and pigments. Arrows refer to connections in the meta-model (Figure 1)

Hypothesis	Arrow	Predictions	Citations
Nutrient availability (the amount of P and the relative availability of N and P) influence competitive interactions between cyanobacteria and other phytoplankton. Loss of N or excessive P relative to N will result in a competitive advantage for N-fixing cyanobacteria Microcystin is an N-rich molecule that appears to be upregulated when there is not enough reduced N, iron, or other trace metals	1	High P relative to N should be associated with higher abundance of N-fixing cyanobacteria and higher concentrations of microcystin	Ginn, Pearson, and Neilan (2010); Oliver et al. (2012); Schindler (2012); Gobler et al. (2016); Omidi et al. (2018); Huisman et al. (2018); Paerl & Otten, 2013, Reynolds, Huszar, Kruk, Naselli-Flores, and Melo (2002), Donald, Bogard, Finlay, Bunting, and Leavitt (2013)
Optimum temperatures for growth of cyanobacteria are higher than many other phytoplankton species. Some taxa have been shown to have greater microcystin production at higher temperatures	2	Warmer water temperatures (corresponding to the warmest months of the year and warmer backwaters) will be associated with higher abundance of cyanobacteria	Paerl and Huisman (2008, 2009); Kosten et al. (2012); Rigosi, Carey, Ibelings, and Brookes (2014); Burford et al. (2020)
Backwater habitats that experience long residence times will be more likely to accumulate larger abundances of cyanobacteria and other phytoplankton	4	Higher water velocity will be associated with lower biovolumes of all phytoplankton, including cyanobacteria	Cloern (2001); Hilton, O'Hare, Bowes, and Jones (2006); Mitrovic, Hardwick, and Dorani (2011); Verspagen et al. (2006); Giblin and Gerrish (2020)
Cyanobacteria have numerous accessory pigments for harvesting solar energy for photosynthesis, many of which are not present in other phytoplankton. These pigments allow cyanobacteria to outcompete other phytoplankton in highly turbid waters. Furthermore, some taxa (such as *Microcystis*) actively regulate their position in the water column to maximize light and nutrient acquisition, which can alleviate light limitation in turbid waters	3	Higher turbidity will be associated with higher biovolumes of cyanobacteria, particular taxa such as *Microcystis* that can actively regulate their buoyancy and low-light adapted taxa such as *Planktothrix*	Dzialowski, Smith, Wang, Martin, and Jr (2011); Scheffer, Rinaldi, Gragnani, Mur, and van Nes (1997); Oliver et al. (2012); Ibelings, Mur, and Walsby (1991); Reynolds et al. (2002)
Emergent and floating vegetation may block most light from reaching phytoplankton. Alternative stable state dynamics (macrophytes compete for nutrients, reduce sediment resuspension, increase sedimentation, provide refuge for zooplankton). Allelopathic exudates from macrophytes suppress cyanobacteria	6	Higher vegetation cover will be associated with lower biovolumes of cyanobacteria	Scheffer (1998); Ibelings et al. (2007); Van Donk and van de Bund (2002); Mohamed (2017)
Depth reduces the near surface concentrations of cyanobacteria due to more sinking cells and less wind-driven resuspension of cells and nutrients; also dilutes the influence of nutrients released from the sediment; shallow water also results in higher water temperature; more propensity of N-limitation in shallow waters to the benefit of N-fixing cyanobacteria	5	Greater depth will be associated with less cyanobacteria abundance and toxicity	Huisman et al. (2018), Scheffer (1998), Descy (1993), Dolman, Mischke, and Wiedner (2016)

(N-fixation; Oliver et al., 2012; Schindler, 2012; Whitton & Potts, 2012) and their unique accessory photopigments and photosynthetic pathways (Oliver et al., 2012). Cyanobacteria also tend to have higher optimum temperatures than other phytoplankton and blooms or high densities of cyanobacteria often occur during mid- to late- summer in systems with periodically recurring blooms (Paerl & Huisman, 2008, 2009; Wynne, Stumpf, Tomlinson, & Dyble, 2010). However, much of the existing data and research has focused on lentic settings, and the unique properties of other ecosystem types may influence environmental controls over both cyanobacteria and other phytoplankton (Cloern, 2001; Hilton et al., 2006). For example, in backwaters and pools of large river systems, cyanobacterial blooms can be regulated by management of water residence time and flushing rates (Baker & Baker, 1981; Cha, Cho, Lee, Kang, & Kim, 2017; Hilton et al., 2006; Mitrovic et al., 2011).

Backwater areas of the Upper Mississippi River (UMR) are central to the biological productivity and diversity observed in the system. These backwater habitats vary in flushing rate, temperature, turbidity, nutrient availability, water depth, and vegetative cover (De Jager & Houser, 2012; Houser et al., 2013) and are critical for limnophilic organisms both as refugia from high water velocity and as reproductive areas (Giblin, 2017; Rutledge, Hupfeld, Gainer, Kim, & Phelps, 2020). Backwaters are essential for fisheries nursery habitat (Sheaffer & Nickum, 1986), fisheries overwintering habitat (Knights, Johnson, & Sandheinrich, 1995), sediment and nutrient assimilation (James, Richardson, & Soballe, 2008), aquatic vegetation production (Burdis, DeLain, Lund, Moore, & Popp, 2020), zooplankton production (Burdis & Hoxmeier, 2011), mussel habitat (Tucker & Atwood, 1995), and waterfowl habitat (Korschgen, Jackson, Muessig, & Southworth, 1987).

Here, we looked at statistical associations between environmental conditions and cyanobacterial abundance and toxicity across eight backwater habitats of the UMR. Because there are many hypothesized environmental drivers of cyanobacterial abundance and toxicity, and because these drivers interact, we compiled these hypothesized drivers into a single meta-model, based on causal modeling principles (Figure 1; Table 1; Grace, Scheiner, & Schoolmaster Jr, 2015).

2 | MATERIALS AND METHODS

2.1 | Study sites

The UMR consists of a series of navigation pools extending from Minneapolis, MN to the confluence of the Ohio River at Cairo, IL. The 27 navigation dams within this area are low-head dams built to maintain sufficient depth in the river for navigation during the low flow season and were designed to have little impact on discharge or water level during high flow and flood conditions (Anfinson, 2003; Sparks, 1995). Navigation pools are unlike reservoirs in that they remain mostly riverine in nature. More detailed descriptions of these contrasting aquatic areas can be found in Strauss et al. (2004).

Data collection took place in eight backwaters between Navigation Pools 5 and 8 of the UMR (Figure 2). Each backwater was sampled monthly from April to October, 2019 ($n = 7$ for each backwater). The earliest sample was April 23 and the final sample was collected on October 15. The eight backwaters sampled were selected to represent a wide range of connectivity to channel inputs. This wide range of connectivity to channel inputs resulted in a wide range of physical and chemical conditions among the eight backwaters sampled. This provided an informative range of limnological conditions to test hypotheses related to drivers of cyanobacterial abundance and cyanotoxin production.

2.2 | Water quality and discharge

Water samples were collected by inverting a two-liter amber bottle at a depth of 0.20 m at each site to assess water column total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), nitrate + nitrite ($NO_3 + NO_2$-N; NO_x), ammonia (NH_3-N), and orthophosphate (PO_4-P) concentrations. Water for phytoplankton enumeration and cyanotoxin analysis were taken from the same water samples. All analyses were performed at the Wisconsin State Laboratory of Hygiene (WSLOH, Madison, WI) utilizing USEPA (United States Environmental Protection Agency) methods. TSS was

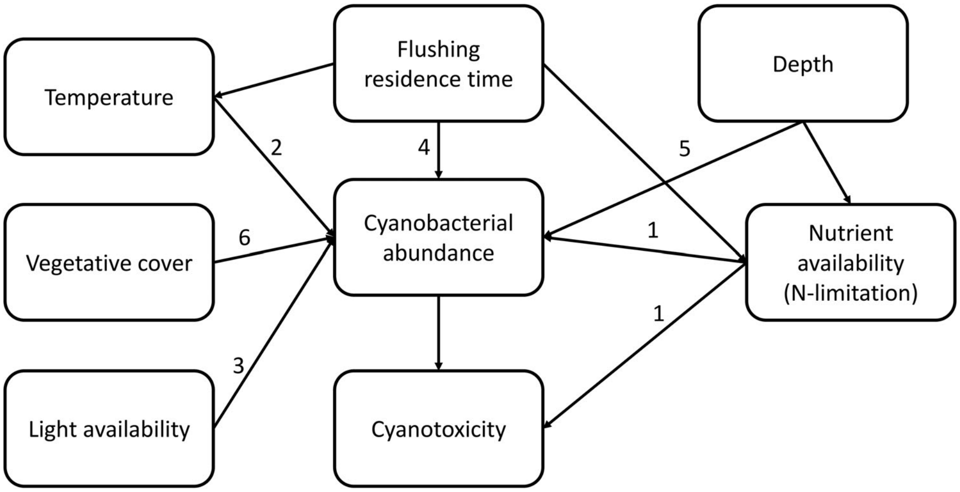

FIGURE 1 Meta-model describing the hypothesized drivers of cyanobacterial abundance and toxicity. Arrow numbers refer to hypothesized connections described in Table 1

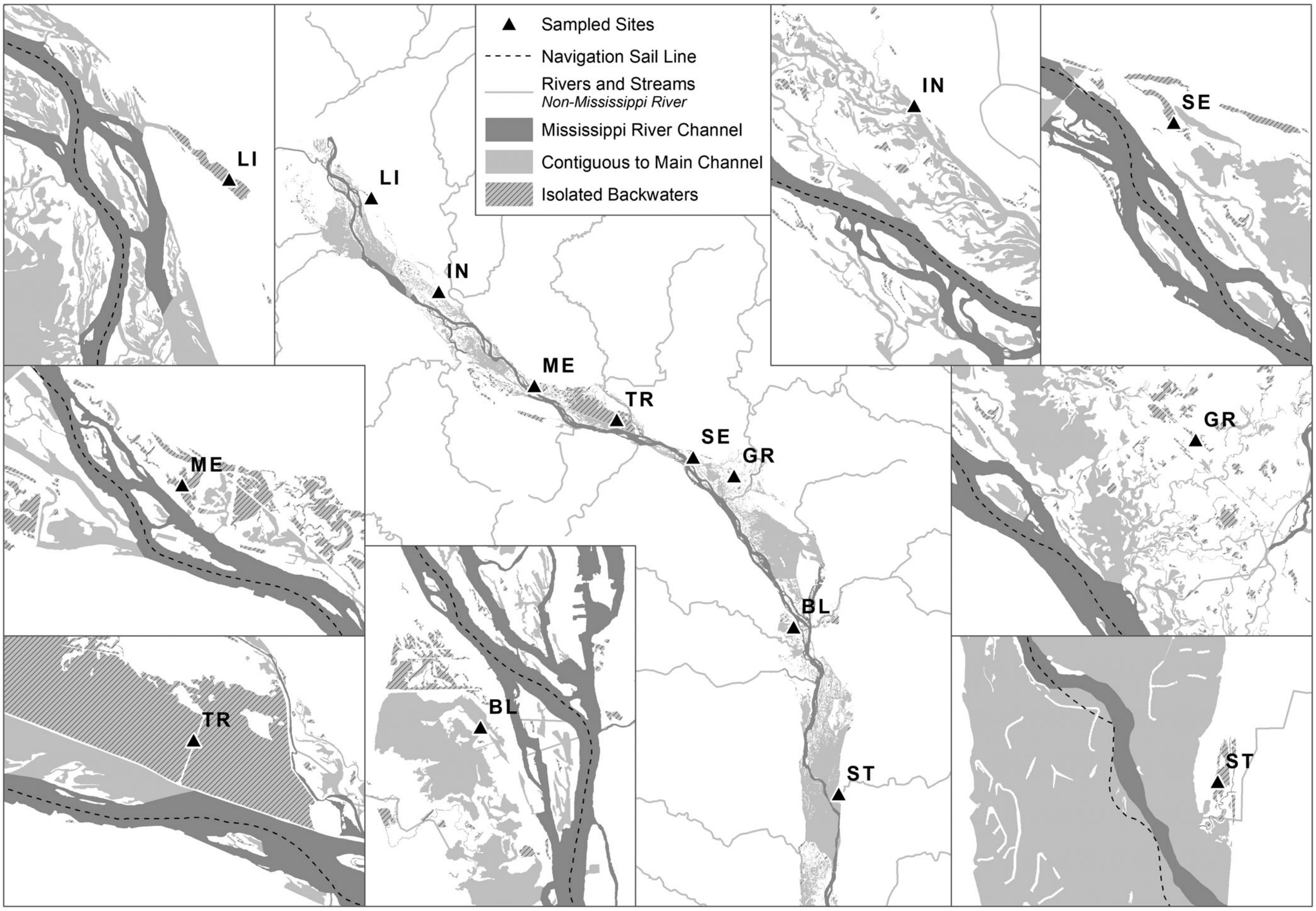

FIGURE 2 Location of study sites within Navigation Pools 5–8 of the Upper Mississippi River. BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge

determined gravimetrically. TN, TP, NO_x, and NH_3 samples were preserved in the field with concentrated H_2SO_4, transported on ice, and refrigerated until analysis. PO_4 samples were transported on ice and refrigerated until analysis. Dissolved inorganic nitrogen (DIN) was calculated as $NO_x + NH_3$. Measurements of water depth (m) and water velocity (m/s; Hach FH950; Hach Company, Loveland, CO) were collected at each site. Water temperature measurements were taken at 0.20 m using a multiparameter sonde (Minisonde MS5; Hach Company). An estimate of vegetation percent cover (emergent, rooted floating, and submersed) was performed within 25 m of the sampling site. Discharge data were collected by the U.S. Corps of Engineers at Lock and Dam 7 at Dresbach, Minnesota and measured in m^3/s.

2.3 | Phytoplankton identification and enumeration

Phytoplankton samples were analyzed at the WSLOH by the Utermöhl sedimentation method. Preserved, well-mixed sample aliquots were settled in Utermöhl chambers and identified and enumerated using a Leica DMIRB inverted microscope at 630× total magnification. A minimum of 300 natural units were enumerated from random, non-overlapping microscope fields with final results expressed as cells/mL and natural units/ml. Cell counts were converted to biovolume (μm^3/ml) using a mixture of prior published data and literature values (Giblin & Gerrish, 2020; Kremer, Gillette, Rudstam, Brettum, & Ptacnik, 2014; Takeda, 1916). Data from past studies of the UMR were used whenever possible. For all remaining genera/species, averages were calculated using published data.

2.4 | Cyanotoxin identification and quantification

Preserved anatoxin-a samples were stored frozen until analysis. Microcystin samples were stored frozen and subsequently underwent three freeze–thaw cycles to lyse cells prior to analysis. Anatoxin-a and microcystin concentrations were determined using 96-well ELISA plate kits (Eurofins Abraxis, Warminster, PA). Samples were analyzed in duplicate according to manufacturer's instructions. In some cases samples were re-run to ensure accurate results. Absorbance at 450 nm was measured using a SpectraMax M5e plate reader (Molecular Devices, San Jose, CA) and a 4-parameter standard curve fit was used to determine the concentration of each sample.

TABLE 2 Correlation coefficients (with bootstrapped 95% confidence intervals) between hypothesized drivers of cyanobacterial abundance and toxicity. Correlation coefficients are estimated using Kendall's tau (recommended for samples with many non-detects). Bold indicates 95% confidence intervals do not include zero

Potential predictors	Cyanobacterial biovolume	N-fixing biovolume	Microcystin concentration	Anatoxin-a concentration
Hypothesis: N-limitation/P-excess				
DIN:PO_4	0.03 (−0.19, 0.24)	−0.09 (−0.31, 0.12)	**−0.23 (−0.42, −0.05)**	−0.11 (−0.31, 0.09)
TN:TP	−0.01 (−0.20, 0.21)	−0.18 (−0.39, 0.03)	**−0.27 (−0.43, −0.11)**	**−0.21 (−0.36, −0.07)**
PO_4	−0.05 (−0.23, 0.13)	−0.17 (−0.35, 0.03)	−0.10 (−0.30, 0.10)	−0.19 (−0.37, −0.02)
TP	0.12 (−0.09, 0.35)	0.08 (−0.18, 0.33)	**0.25 (0.04, 0.46)**	**0.23 (0.02, 0.45)**
DIN	−0.03 (−0.22, 0.15)	**−0.22 (−0.41, −0.03)**	**−0.36 (−0.51, −0.20)**	**−0.29 (−0.44, −0.16)**
TN	0.17 (−0.02, 0.36)	−0.001 (−0.23, 0.23)	−0.08 (−0.31, 0.15)	0.10 (−0.11, 0.32)
Hypothesis: Temperature				
Water temperature (°C)	0.12 (−0.08, 0.33)	**0.40 (0.26, 0.55)**	**0.22 (0.02, 0.42)**	0.17 (−0.02, 0.36)
Hypothesis: Flushing				
Water velocity (m/s)	−0.05 (−0.25, 0.14)	**−0.18 (−0.36, −0.004)**	**−0.43 (−0.52, −0.34)**	**−0.22 (−0.31, −0.13)**
Hypothesis: Turbidity				
TSS	0.15 (−0.07, 0.37)	0.06 (−0.21, 0.33)	**0.25 (0.02, 0.49)**	**0.39 (0.24, 0.55)**
Hypothesis: Vegetative shading/competition for nutrients/allelopathy				
Vegetative cover (%)	−0.11 (−0.34, 0.11)	0.08 (−0.16, 0.33)	−0.12 (−0.38, 0.13)	**−0.34 (−0.51, −0.20)**
Hypothesis: Shallow depth results in more wind resuspension, increased N-limitation and higher water temperature				
Water depth	−0.14 (−0.33, 0.06)	**−0.35 (−0.53, −0.17)**	**−0.34 (−0.53, −0.15)**	**−0.26 (−0.42, −0.11)**

High phosphorus loads are often associated with higher likelihood of cyanobacterial dominance and cyanobacterial blooms (Schindler, Carpenter, Chapra, Hecky, & Orihel, 2016). This is hypothesized to occur because as phosphorus increases in availability, non-cyanobacterial taxa become N-limited, while cyanobacteria can “fix” atmospheric N^2 gas into biologically useful forms. However, not all cyanobacterial taxa have the genes necessary to perform N-fixation. Of the 12 cyanobacterial taxa identified in our study, four (*Dolichospermum* spp., *Aphanizomenon* spp., *Cylindrospermopsis raciborskii*, and *Pseudanabaena* spp.) appear to include some species that have previously been identified as having the ability to fix nitrogen (Bergman, Gallon, Rai, & Stal, 1997; De Nobel, Huisman, Snoep, & Mur, 1997; Osburn, Wagner, & Scott, 2021; Willis, Chuang, & Burford, 2016). Because N-fixing taxa would respond most strongly to the hypothesized nutrient effects, and because these taxa can also produce microcystin and other toxins, we looked at the response of these taxa separately as part of the analysis (Rantala et al., 2004).

2.5 | Data analysis

All statistics were performed in R version 3.6.1 (R Development Core Team, 2019). Simple correlations between variables were estimated using Kendall's τ, as this non-parametric method is preferred when using data containing non-detects (Helsel, 2005). Estimates of 95% confidence intervals were created using bootstrapping (1,000 iterations). Associations between 11 potential predictors and four cyanobacterial response variables were estimated using this approach (Table 2).

Structural equation models were estimated using local estimation methods, which allows for flexibility in model form (Grace et al., 2015). This was done to model microcystin concentration as present/absent (using logistic regression), because the small number of observations above the detection limit made it difficult to draw strong inference about the numerical value of the microcystin concentration. Estimates of R^2 for the logistic regression (i.e., for the portion of the model relating to microcystin presence/absence) were made using the approach described in Nakagawa, Johnson, and Schielzeth (2017). The structural equation model format assumes that samples were independent. Considering the time scale of the sampling (monthly) and lifespans of phytoplankton (hours to days), and presuming measured environmental conditions were representative of the important differences among sites and time, it was considered that this was a reasonable exploratory approach. Within individual sites, N-fixing biovolume data collected 1 month apart had correlation coefficients that ranged between −.19 and .37.

To convert our meta-model (Figure 1) into a structural equation model, nutrient characteristics were simplified. To assess covariation in the nutrient data, we used principal components analysis (PCA; Manly, 2005). The PCA was done using the “prcomp” function in base R. Data were scaled (subtract the mean and divide by the standard deviation) prior to analysis.

3 | RESULTS

3.1 | Spatio-temporal cyanobacterial abundance and toxicity

River discharge in 2019 was greater than the long-term (1980–2019) median during all seven sampling events that ranged from April 23 to October 15 (Figure 3). Cyanobacteria were never the dominant phytoplankton in terms of biovolume in our study sites (Figure 4). Among study sites, cyanobacterial biovolume was greatest in Trempealeau National Wildlife Refuge (TR; Figure 5, note log scale), and was greatest in August and September. These patterns were very similar when looking at just the N-fixing taxa (Figure 5). Microcystin concentrations above the detection limit were observed in 20 samples (of the 56 total samples collected throughout the study), and the seven highest concentrations were in TR. At times during late summer, concentrations were greater than the WHO (World Health Organization) drinking water-quality standard (Figure 5). Microcystin concentration

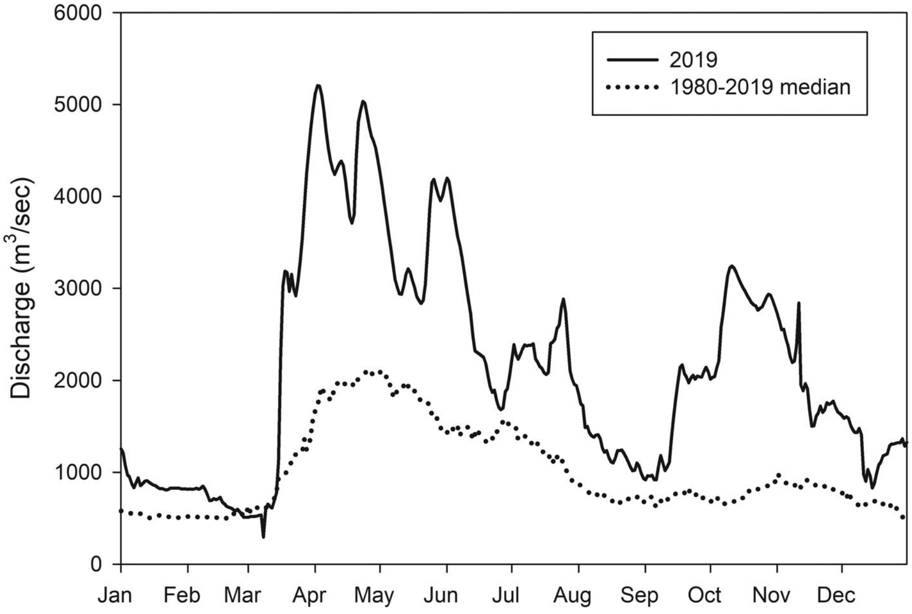

FIGURE 3 Discharge (m^3/s) at Lock and Dam 7 during 2019. The long-term median (1980–2019) is denoted with the dotted line. Discharge data were from https://www.mvp-wc.usace.army.mil

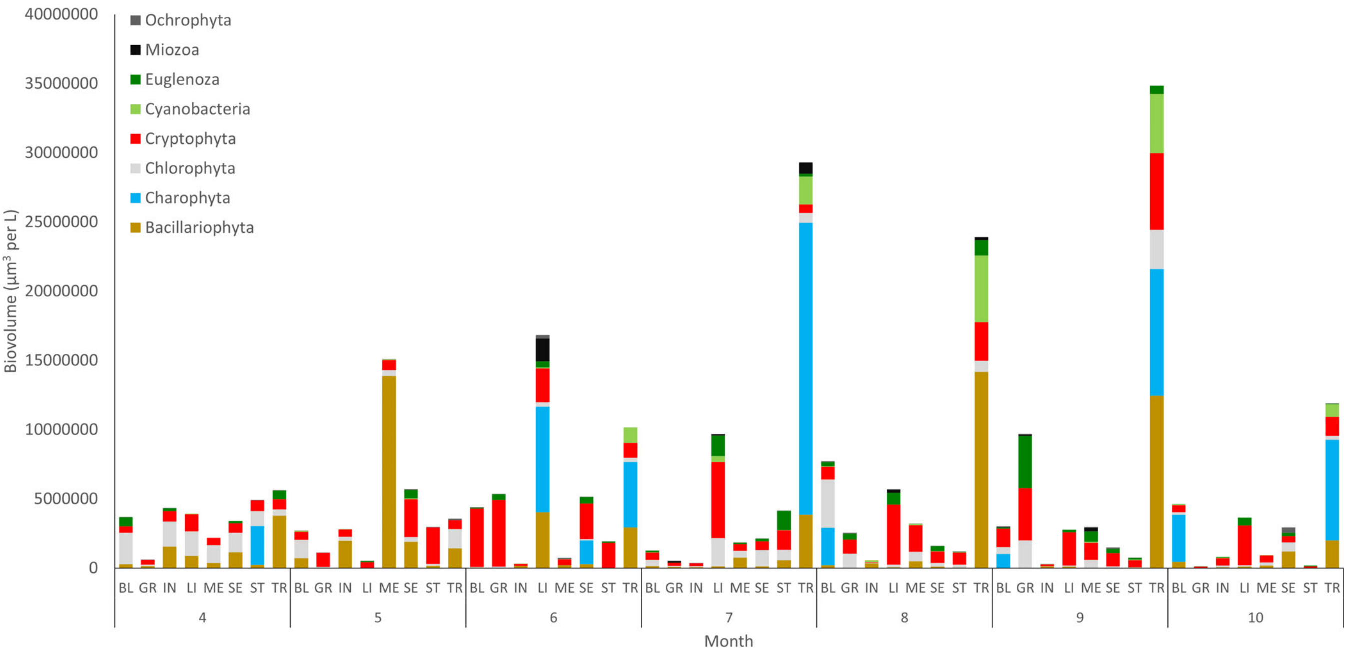

FIGURE 4 Phytoplankton biovolumes by major taxonomic groups in backwater habitats of the Upper Mississippi River during the 2019 growing season. BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge [Color figure can be viewed at wileyonlinelibrary.com]

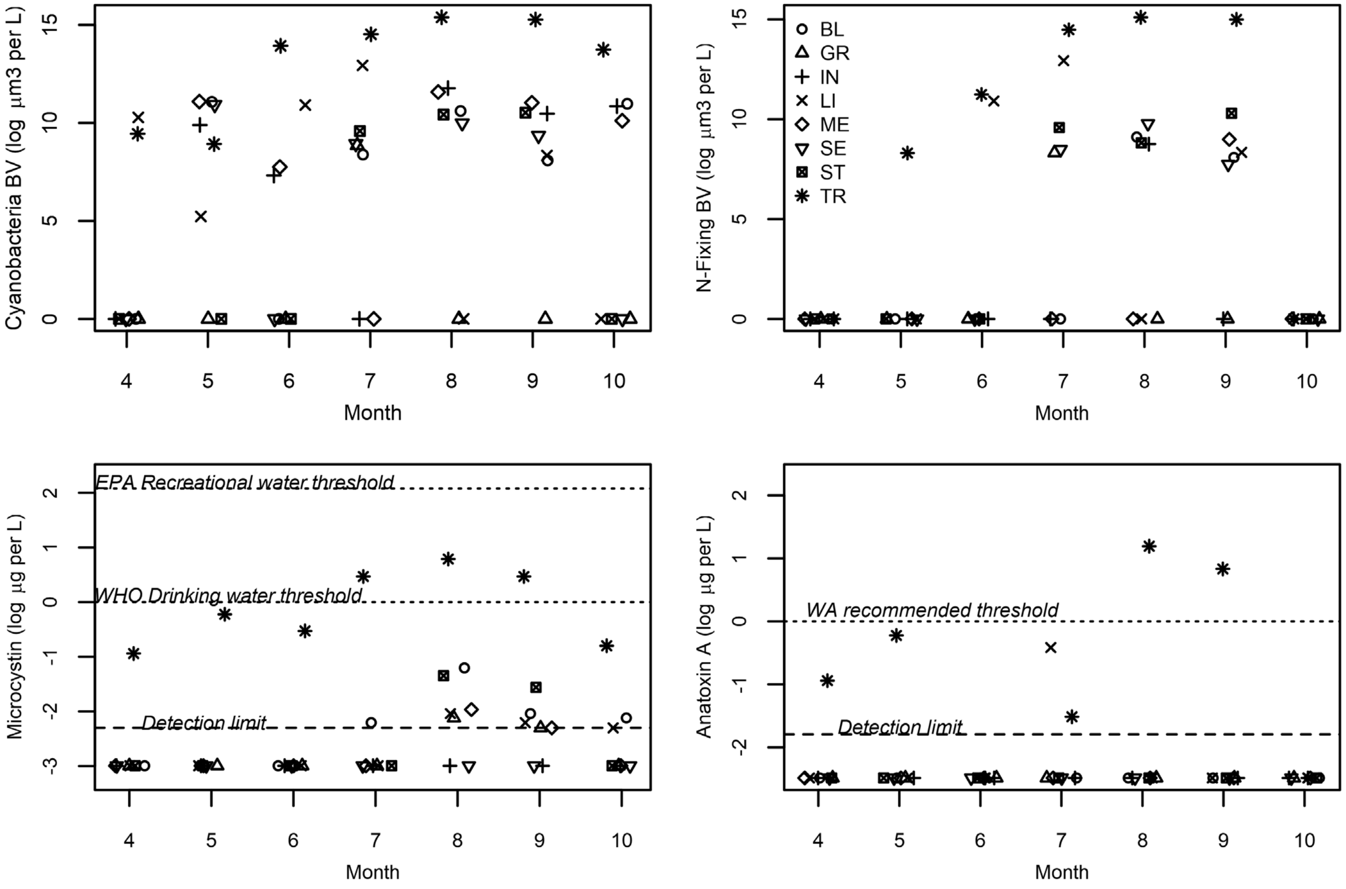

FIGURE 5 Cyanobacteria biovolume, N-fixing cyanobacteria taxa biovolume, microcystin concentration and anatoxin-a concentration by backwater and month. BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge. The WHO drinking water standard, USEPA recreational water recommendation for microcystin, and Washington State (USA) recommended threshold for anatoxin-a are represented with dotted lines. The detection limits are represented with the dashed lines

was moderately associated with cyanobacterial biovolume (Kendall's $\tau = .35$ [.14, .56], 95% confidence intervals) and the biovolume of potential N-fixing cyanobacteria ($\tau = .38$ [.14, .63]). Anatoxin-a occurred in only six samples (once in Lizzy Paul's Pond; LP and the rest in TR). Anatoxin-a was also positively associated with cyanobacterial biomass ($\tau = .34$ [.13, .54]) and the biovolume of potential N-fixing cyanobacteria ($\tau = .41$ [.17, .68]). Two TR samples exceeded the provisional anatoxin-a guidance developed for the state of Washington (the only regulatory body that appears to have developed guidance in the USA; Hardy, 2008; Figure 5).

3.2 | Univariate associations between environmental conditions and cyanobacterial abundance and toxicity

Individual environmental characteristics that were representative of our core hypotheses (Table 1) were not strongly correlated with total cyanobacterial biovolume across our sites (Table 2). However, the hypotheses about the importance of nutrients (either directly as in hypothesis 1 or indirectly in hypothesis 4 and 5) are likely to be more influential on specifically the N-fixing taxa in the cyanobacterial community. N-fixing cyanobacterial biovolume was negatively correlated with DIN, water depth, and water velocity, and positively correlated with water temperature (Table 2; Figure 6).

Microcystin concentrations were strongly associated with many of the same environmental variables as the N-fixing biovolume (Table 2), but also were positively correlated with TP and TSS and negatively correlated with the molar ratio of DIN:PO_4 and TN:TP (Figure 7). Anatoxin-a and microcystin concentrations had similar univariate associations with environmental data, except that anatoxin-a was not strongly associated with DIN:PO4 or water temperature, and was negatively correlated with rooted vegetation cover (Table 2; Figure 8). Water velocities seemed to have a major effect on cyanotoxins. Neither of the cyanotoxins measured here were ever observed above the detection limit in backwaters with measurable water velocities (Figures 7 and 8).

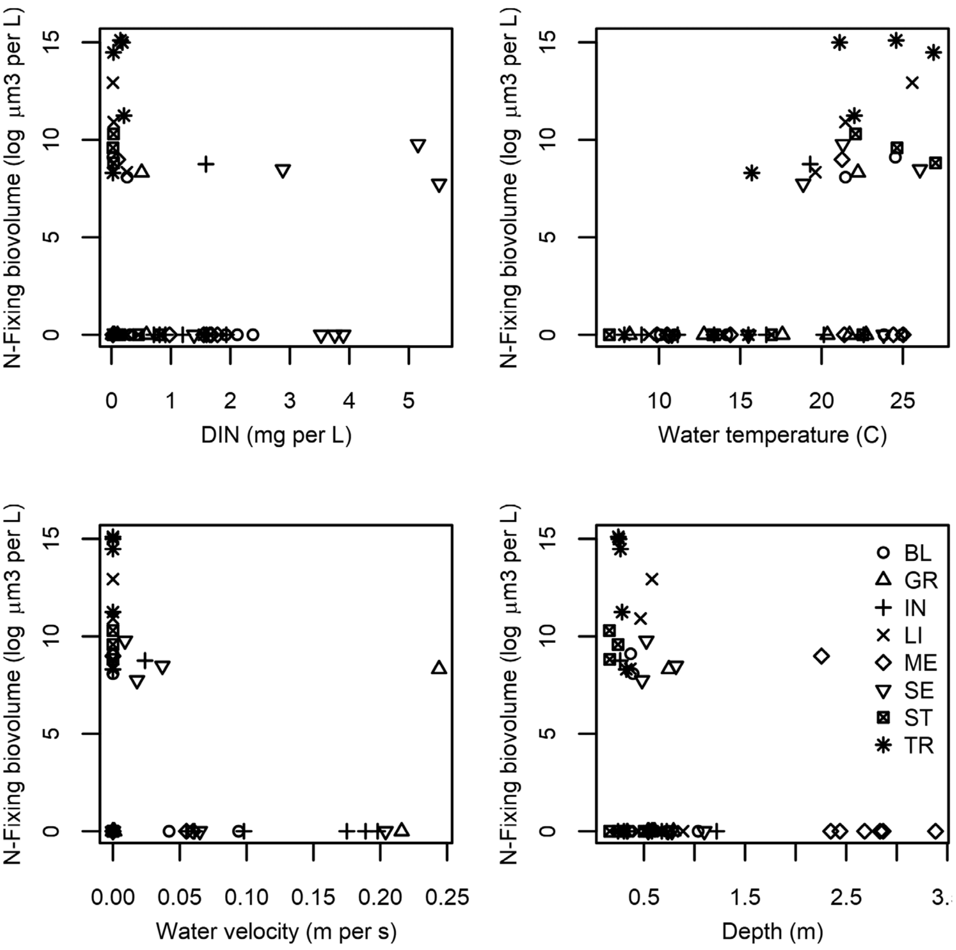

FIGURE 6 Strong associations between N-fixing cyanobacterial taxa and environmental conditions in backwater habitats of the Upper Mississippi River. N-fixing taxa included *Aphanizomenon* spp., *Dolichospermum* spp., *Pseudanabaena* spp., and *Cylindrospermopsis raciborskii*., BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge. Note that all vertical axes are log-transformed

3.3 | Structural equation models of multiple hypothesized interactions between environmental factors and cyanobacterial abundance and toxicity

Combining many of our hypotheses into a structural equation model required reduction in the number of variables under consideration due to sample size limitations (Grace, 2006). The most prominent example of this was in the nutrient conditions. Using PCA, we identified one axis of variation that accounted for most of the among-sample variation in nutrient conditions (PCA 1, 0.52 proportion of variation explained; Table 3). This axis of variation was most strongly correlated with DIN, and DIN had the strongest univariate correlation with cyanobacterial abundance and toxicity among the nutrient variables. DIN concentration is also strongly related to our hypothesis about the importance of nitrogen availability to cyanobacterial abundance and toxicity (Table 1). For these reasons, we used DIN concentration as our representation of nutrient availability.

The combination of our hypotheses into a structural equation model suggests that some hypothesized mechanisms were more important and some were less important than we might otherwise suspect based on univariate relationships (Figure 9). The only two environmental conditions that had strong correlations with N-fixing cyanobacterial biovolume were TSSs and water temperature, although water temperature is itself strongly influenced by water velocity, a surrogate for flushing (Figure 9). Overall, the R^2 values for the N-fixing biovolume and the presence of microcystin suggest that a moderate amount of variation was being explained by this model (Figure 9). Our hypotheses related to the direct effects of depth and DIN availability were not supported. We did explore using other metrics for nutrient availability, but these metrics did not qualitatively alter the results (not shown here).

4 | DISCUSSION

A substantial range of total cyanobacteria and N-fixing cyanobacteria biovolume as well as microcystin and anatoxin-a concentrations were observed among the study backwaters. The range observed among these harmful algal bloom endpoints were the result of the large range of environmental conditions among the eight backwaters. The analysis here finds support for many hypothesized environmental drivers of high cyanobacterial biomass (low DIN, low turbulence, low flushing, light availability, and warm water temperatures; Paerl & Otten, 2013). These environmental drivers often co-vary, making it difficult to distinguish which is causative, but structural equation modeling suggested that variation in backwater cyanobacterial biomass was driven mostly by physical factors (light and temperature) and cyanotoxin concentrations were related more with cyanobacterial biomass and nutrient availability.

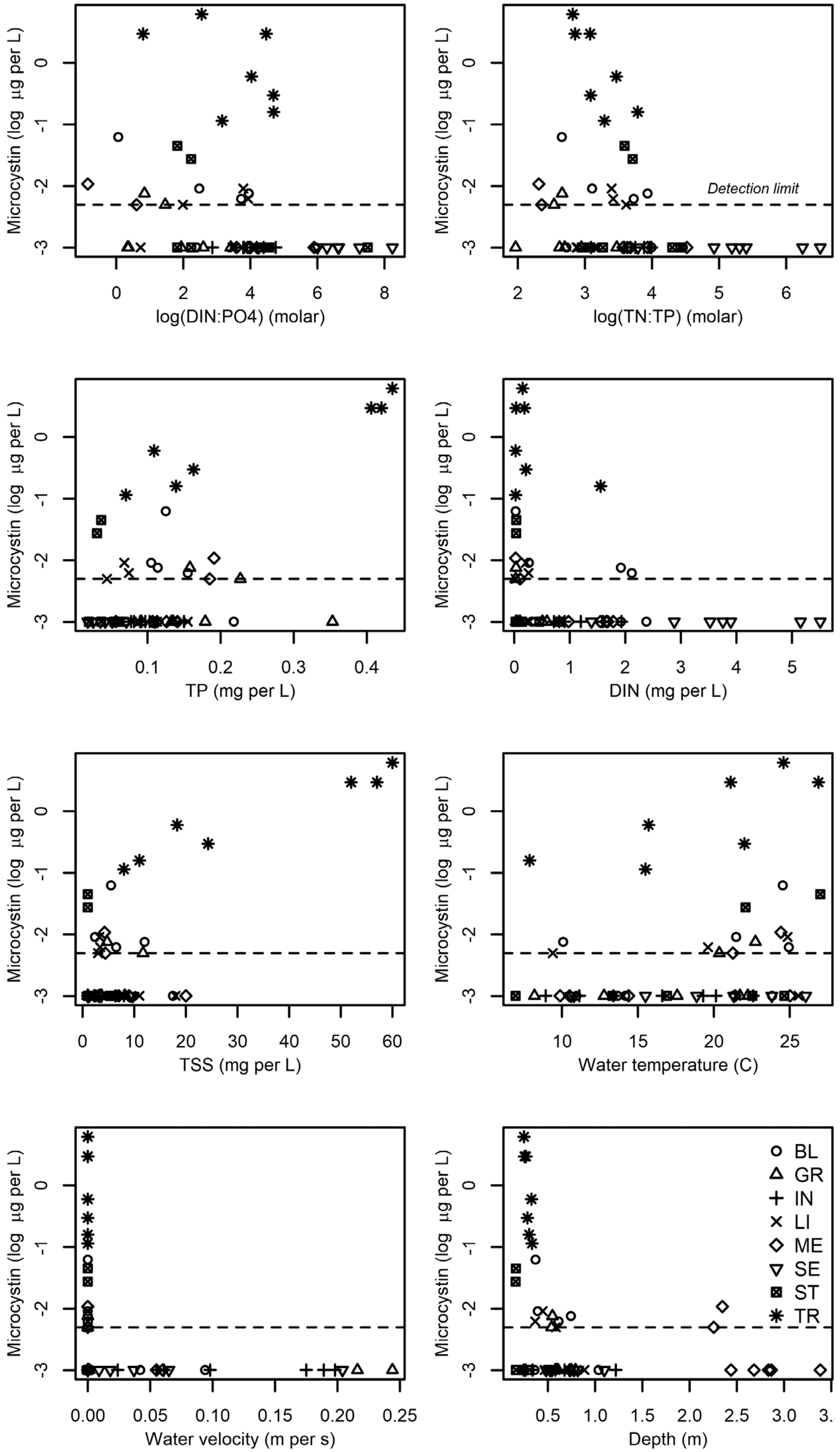

FIGURE 7 Strong associations between microcystin concentration and environmental conditions in backwater habitats of the Upper Mississippi River. BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge. Note that all vertical axes are log-transformed

Vigorous debate regarding the role of N versus P in driving CyanoHABs (cyanobacterial harmful algal blooms) has occurred over the past several decades (Gobler et al., 2016; Schindler et al., 2016). In recent years, evidence has accumulated pointing to species-specific responses to differential levels of N versus P loading (Donald et al., 2013; Rigosi et al., 2014). In our data, low DIN was associated

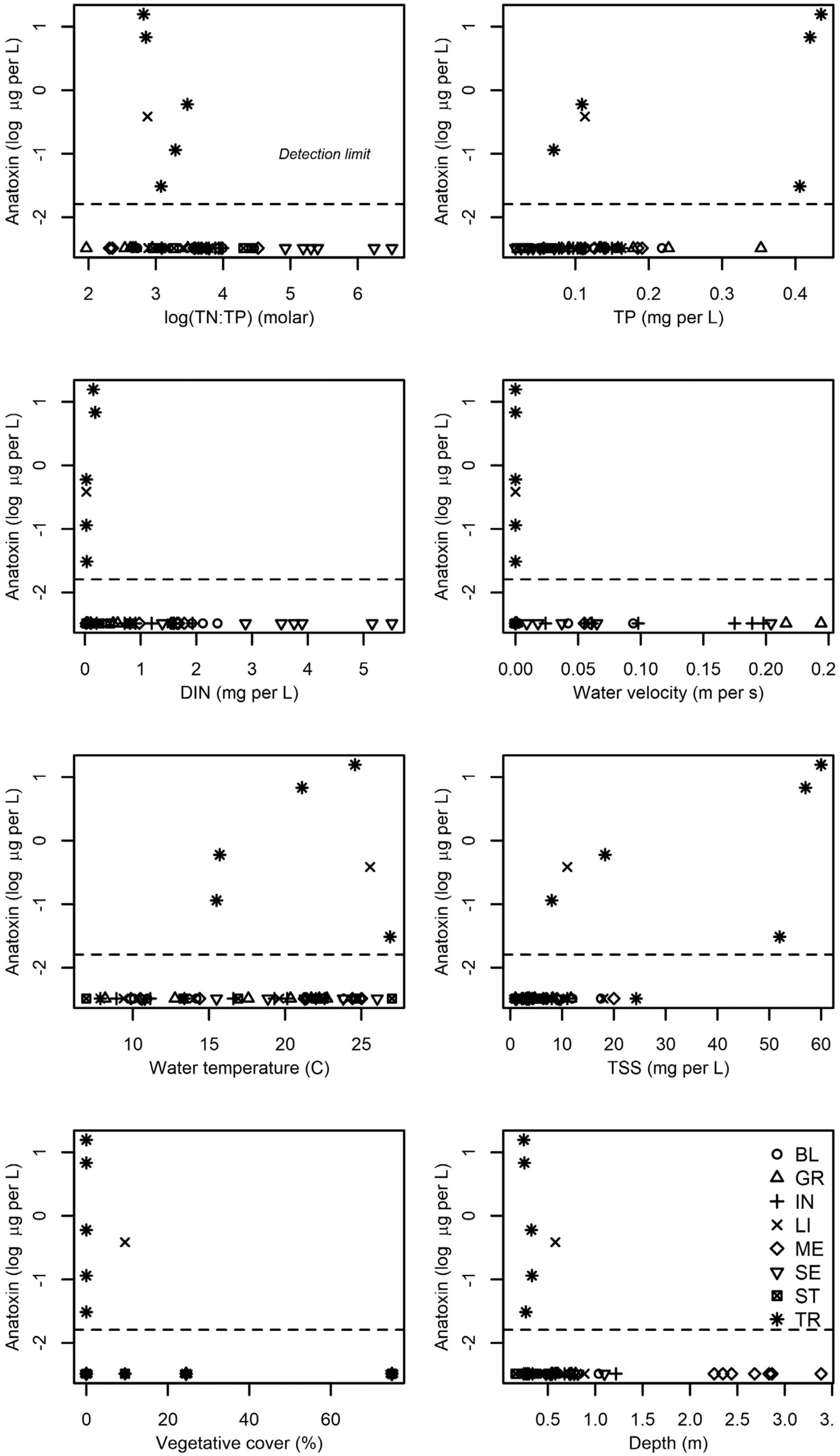

FIGURE 8 Strong associations between anatoxin-a concentration and environmental conditions in backwater habitats of the Upper Mississippi River. BL, Blue Lake; GR, Great River Backwater; IN, Indian Slough; LI, Lizzy Paul's Pond; ME, Mertes Lake; SE, Second Lake; ST, Stoddard Backwater; TR, Trempealeau Wildlife Refuge. Note that all vertical axes are log-transformed

with increases in cyanobacteria biovolume (especially N-fixing cyanobacteria) and microcystin and anatoxin-a production. High TP was positively associated with microcystin and anatoxin-a production. This result may be somewhat atypical due to the unusually high discharge that occurred during 2019. High discharge correlates with elevated N concentration among Mississippi River backwaters, and this may have

TABLE 3 Principal components analysis (PCA) of spatial and temporal variation in nutrient conditions in backwaters of the Upper Mississippi River. Loadings are the simple Pearson's correlation coefficients between the data and the PCA axis. Axes that explained less than 10% of the variation are not shown. All data were scaled prior to analysis

	PCA axis 1	PCA axis 2	PCA axis 3	PCA axis 4
Proportion of variation explained	0.52	0.21	0.14	0.10
Parameters	**Loadings**			
DIN:PO_4	0.40	−0.15	−0.30	−0.83
TN:TP	0.51	0.02	0.11	0.40
DIN	0.54	0.17	0.21	0.03
TP	−0.22	0.62	−0.64	0.06
TN	0.48	0.39	−0.20	0.15
PO_4	−0.15	0.64	0.63	−0.36

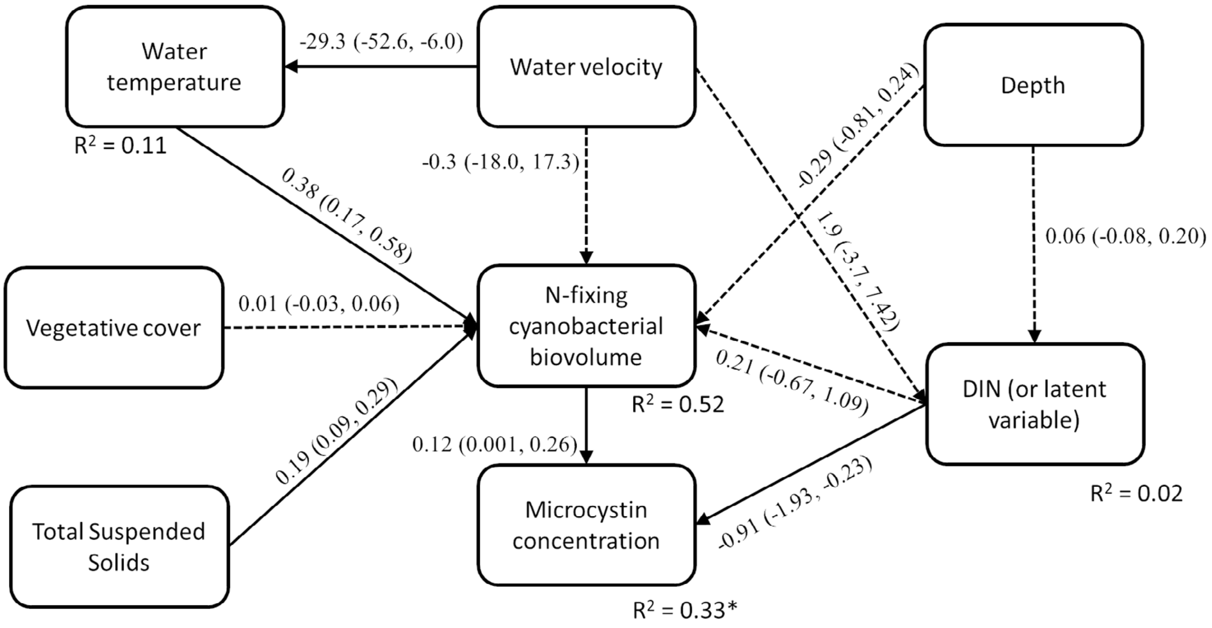

FIGURE 9 Structural equation model showing relationships between hypothesized environmental factors driving variation in cyanobacterial abundance and toxicity in backwaters of the Upper Mississippi River during the 2019 growing season. DIN refers to dissolved inorganic nitrogen. Dashed lines had parameter estimates that overlapped zero. Model was parameterized using local estimation, see methods for details of analytical relationship. Parameter estimates are unstandardized, however, N-fixing cyanobacteria were log-transformed and microcystin concentration was modeled using a logistic regression for above or below the detection limit (positive values indicate above the detection limit). *Indicates that this is a pseudo-R^2 estimated using methods described in Nakagawa et al. (2017)

exacerbated differences in N among backwaters in this study (Houser & Richardson, 2010). While the evidence related to the linkage between elevated N and CyanoHAB endpoints may be lacking from this study, other eutrophication problems such as excessive free-floating plant production have been linked with excessive N loading to Mississippi River backwaters (Giblin et al., 2014).

Loss of water depth via sedimentation in backwaters has been a source of public concern since the dams were constructed on the UMR in the 1930s. Much of the sediment delivered to backwaters occurs during large flood events (Theis & Knox, 2003). This is of concern because Mississippi River discharge continues to increase (Zhang & Schilling, 2006). Our finding of increasing N-fixing cyanobacteria and microcystin and anatoxin-a production with shallower water depths is germane to river management. As backwaters become shallower, the potential for CyanoHABs could increase. The effects may, however, be indirect as shallower waters tend to be warmer, keep cyanobacteria resuspended via wind, have an increased tendency toward N-limitation, and are more likely to be overwhelmed by large nutrient release from the sediments (Dolman et al., 2016; Noges, 2009; Scheffer, 1998; Winder & Sommer, 2012). Preservation and restoration of backwater habitat will be essential for a resilient ecosystem.

Mississippi River backwaters are known to alternate between clear/macrophyte- and turbid/phytoplankton-dominated states (Burdis et al., 2020; Giblin, 2017). A variety of mechanisms can contribute to the development and persistence of the clear water state ranging from macrophytes blocking underwater light, macrophytes reducing resuspension of sediment and nutrients, competition for available nutrients, trophic cascades, and production of allelopathic exudates that suppress phytoplankton (Giblin, 2017; Ibelings et al., 2007; Mohamed, 2017; Scheffer, 1998; Van Donk & van de Bund, 2002). We observed anatoxin-a in backwaters that had low macrophyte abundance, indicating that efforts to transform more river reaches to the clear/macrophyte state which may also reduce potential cyanotoxin production.

Mississippi River backwaters experience a wide range of connectivity to channel inputs of sediment and nutrients. Factors like the size, orientation and number of inlets/outlets, backwater size and shoreline complexity can affect the connectivity of a backwater. With differing connectivity, water velocity and residence time (a proxy for flushing) change. Water residence time can have profound effects on a variety of ecological processes including nitrogen uptake/dynamics (Cloern, 2001; James et al., 2008), macrophyte versus phytoplankton dominance (Hilton et al., 2006), and species-specific responses of phytoplankton (Giblin & Gerrish, 2020). Modifying residence time is one of the most predictable methods to improve water quality and aquatic habitat and thus is of interest to, and a tool utilized for, habitat restoration and enhancement on the UMR (Houser et al., 2013; James et al., 2008; Miranda, 2005). Flushing has even been utilized as a successful mitigation tool to control cyanobacteria production (Mitrovic et al., 2011; Verspagen et al., 2006). Our results showed that lower water velocity (our proxy for flushing) resulted in elevated N-fixing cyanobacteria biovolume and cyanotoxin concentrations. Many backwater complexes are currently isolated via manmade levees from potential flushing and thus water quality could be improved by reconnecting some of these artificially isolated habitats to channel inputs (Giblin & Gerrish, 2020).

Total suspended solids concentration was one of two environmental variables showing direct influence over N-fixing cyanobacteria biovolume in our structural equation modeling. TSSs are a proxy for water column turbidity and available underwater light. Many cyanobacteria species have gas vesicles that allow the regulation of buoyancy to harvest light (Walsby, 1994). Once at the surface, cyanobacteria can outcompete other phytoplankton for light under low turbulence conditions (Huisman et al., 2004). Cyanobacteria also have specific adaptations related to photoacclimation and photoadaptation that allow for light harvest at low underwater light and prevent photodamage by high irradiance near the surface (Oliver et al., 2012). At extreme turbidity, cyanobacteria can begin to be light limited, but at conditions beyond those observed in our study (Dzialowski et al., 2011). Our results showed increased CyanoHAB endpoints among backwaters with higher TSSs suggesting that cyanobacteria are able to outcompete, by shading out, and are better adapted to low and extreme light than other phytoplankton.

Water temperature was another environmental variable showing direct influence over N-fixing cyanobacteria biovolume in the structural equation modeling. The optimal temperature for growth of cyanobacteria is higher than other phytoplankton (Burford et al., 2020; Paerl & Huisman, 2008, 2009). The relative importance of temperature tends to be species specific, with certain taxa like *Microcystis*, showing an increased affinity for warmer water temperatures (Giblin & Gerrish, 2020; Rigosi et al., 2014). The positive relationship found in our study between increasing water temperature and increasing N-fixing cyanobacteria, microcystin, and anatoxin-a is concerning given future warming projections (Wells et al., 2020). More days with water temperatures over 20°C and higher peak summer temperatures are likely to increase bloom severity (Reynolds, 2006). Severe winters required to reduce winter inoculum of cyanobacteria are also likely to become less prevalent (Reeders, Boers, Van der Molen, & Helmerhorst, 1998).

Local natural resource management agencies address their conservation goals within the context of larger environmental changes such as climate change and excessive nutrient loading. Thus, there is a need to pursue local adaptive actions to lessen the impacts of these larger environmental trends. Warm water temperatures were clearly linked to higher cyanobacterial biovolumes in our study. The linkage between low water velocity (flushing) and high temperatures presents one way to adapt to climate-induced CyanoHAB impacts. Of the eight study sites, the Trempealeau Wildlife Refuge site was the most impaired with regard to CyanoHABs (highest cyanobacteria biovolume, N-fixing cyanobacteria biovolume, microcystin, and anatoxin-a concentrations). This site was unique in that it is isolated from channel inputs via a manmade levee. The unique isolated condition of this area helped to create a confluence of environmental conditions uniquely suited to CyanoHAB impairment (high phosphorus, low DIN, low N:P, low turbulence, low flushing, and warm water temperature). Reconnecting this >2,500-ha complex, and others like it, with the Mississippi River are near-term adaptations that could be initiated as society attempts to address longer range problems related to carbon emission and excessive nutrient loading.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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