

1 **Total Phosphorus trends in Mississippi and Atchafalaya River Basin Watersheds:**
2 **Exploring the roles of streamflow and watershed features 2000-2020.**

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16 1. Abstract

17 A consistent method of analyzing riverine phosphorus concentrations and load trends across
18 subbasins of the Mississippi Atchafalaya River Basin (MARB) can indicate whether and where
19 nutrient loads and concentrations have increased or decreased over the long-term. This can
20 help evaluate the success of federal and state nutrient reduction plans being implemented
21 across the landscape and inform adaptive management of load reduction practices. In this
22 study, water quality and streamflow monitoring stations meeting common criteria within sub-
23 watersheds of the MARB were identified and flow normalized (FN) total phosphorus (TP) yield
24 and concentration trends for the period of 2000-2020 were computed through the Weighted
25 Regression on Time, Discharge, and Season (WRTDS), which allowed estimation of streamflow
26 and non-streamflow components of trends. Of the 132 TP sites meeting screening criteria for
27 load calculations and trend analysis, 33.3% and 50.8% had likely increases and decreases of
28 FN concentrations, respectively, while FN yield showed a nearly opposite distribution of trends:
29 54.5% likely increases and 28.8% likely decreases. Watersheds dominated by urban and
30 cultivated cropland tended to have high FN TP concentrations and yields, and many of the
31 urban dominated watersheds had decreasing yield trends. Factors other than streamflow were
32 dominant for 74.2% and 39.4% of sites for concentration and yield trends, respectively. Trends
33 were weakly correlated with land cover and other watershed variables. Identifying causal factors
34 for trends probably requires finer scale analysis of individual watersheds.

35 **Keywords:** Nutrient trends, Streamflow trend component, non-stationary flow normalization,
36 Weighted Regressions on Time, Discharge, and Season (WRTDS) model.

37 2. Introduction

38 Elevated phosphorus (P) loads in surface waters were generally issues of local and regional
39 concern during the 1960s and 1970s (Schindler et al. 2016). In the late 1990s, recognition that
40 nutrients reaching the Gulf of Mexico were contributing to extensive benthic hypoxia expanded
41 regional concerns to the entire Mississippi Atchafalaya River Basin (MARB) (Rabalais et al.
42 1996; Rabalais and Turner 2019). Covering more than 40% of the continental US, the MARB
43 includes intensive and highly productive agricultural areas (Goolsby and Battaglin, 2001).
44 Riverine P, stemming from fertilizer/manure inputs, municipal and industrial wastewater, stream
45 channel erosion and legacy P sources (Stackpoole et al., 2019; Schmadel et al., 2024),
46 discharge to the Gulf of Mexico partly carried by suspended particles and contribute to
47 eutrophication, algal blooms, and hypoxia processes (Jickells, 2005; Adhikari et al., 2015).

48 Strategies to reduce the amount of riverine nutrients have been implemented at federal, state,
49 and local agencies (Mississippi River Gulf of Mexico Watershed Nutrient Task Force, 2008).
50 Compilation of long-term changes in P loads and concentrations across the MARB allows the
51 evaluation of the effectiveness of these management practices implemented at various scales
52 along with other factors that influence riverine P, such as changes in hydrology and land cover.
53 To evaluate the changes of nutrient loads occurring through decades and distinguish the
54 impacts of random year-to-year streamflow variations from long-term trends, the Weighted
55 Regression on time, discharge, and season (WRTDS) was developed (Hirsch et al., 2010;
56 Kreiling and Houser, 2016; Oelsner et al., 2017; Murphy and Sprague, 2019). A further
57 development of the WRTDS tool allowed the computation of the impacts of streamflow non-
58 stationarity on nutrient trends (Hirsch, 2018; Choquette et al., 2019). This upgrade enabled the
59 separation of flow driven components of trends from all other factors that could impact flow

60 normalized (FN) nutrient concentration and load trends (Murphy and Sprague, 2019), with the
61 latter hereafter labeled non-streamflow component.

62 The WRTDS has been widely implemented to evaluate the long-term changes of P in the MARB
63 at various spatial and temporal scales. Kreiling and Houser (2016) used WRTDS to evaluate the
64 long-term trends of total P (TP) in six upper tributaries of the MARB finding declining FN
65 concentrations in four rivers for the 1991-2014 period. Oelsner et al. (2017) compiled several
66 constituents of water quality data from multiple agencies and conducted nutrient trends
67 analyses for rivers and streams throughout the USA between 1972 and 2012 and results were
68 synthesized by Murphy and Sprague (2018) showing that TP FN concentration trends were
69 relatively insensitive to changes in streamflow, while FN load trends were somewhat more
70 sensitive streamflow while also sensitive to other factors. However, in this study the influence of
71 watershed characteristics on trends was not assessed. The National Water Quality Network (US
72 Geological Survey, 2024) conducted a similar trend analyses for 110 USGS water quality
73 monitoring sites across the U.S through 2022 although a synthesis of these results has not yet
74 been conducted.

75 Nutrient availability and advective flow through watershed compartments are the main factors
76 controlling the fate and transport of these pollutants (Speir et al., 2021). Both streamflow and
77 non-streamflow components are influenced by basin features. Topography, soil, land use, and
78 climate, determine flow processes such as rainfall-to-runoff transition, infiltration, and
79 evapotranspiration (Goyette et al., 2019). Current and historical land use influence the non-
80 streamflow component, establishing where fertilizer and legacy phosphorus could be present,
81 as well as the location of point sources from urban wastewater discharge (Sharpley et al., 2013;
82 Schmadel et al., 2024). Furthermore, the basin area determines the complexity of the studied
83 hydrological system. In basins larger than about 200 square kilometers, various heterogeneous

84 interconnected systems regulate the fate and transport of nutrients, as opposed to small
85 drainage basins where nutrient sources and transport paths can be clearly identified (Alexander
86 et al., 2002).

87 In this paper we conduct a methodologically consistent trend analysis of riverine TP
88 concentration and yield (load/drainage area) across the MARB for the 2000-2020 period,
89 estimating the relative influence of streamflow and non-streamflow components on trends. For
90 the analysis, a harmonized and screened dataset of TP concentration and streamflow records
91 was compiled. Finally, trend results and trends component influences were correlated with basin
92 features (i.e., drainage area, relief, upstream dam storage, and dominant land use).

93 Understanding how various factors influence P trends across the diverse subbasins within the
94 MARB enables a fair assessment of applied environmental nutrient reduction policies by
95 understanding the possible impacts that long-term flow variations could have on nutrient trends.
96 Moreover, it would facilitate the projection of water quality trends under future possible
97 scenarios (Murphy and Sprague, 2019). An analogous analysis was performed for nitrate-N
98 (Botero-Acosta et al., 2025).

99 3. Materials and methods

100 The 20-year trend period (2000-2020) was chosen for this study with 2-year additional periods
101 at the starting and ending periods to improve the accuracy of WRTDS load estimations of 2000
102 and 2020 loads. Preliminary analyses revealed that this 20-year trend period provided a
103 sufficiently large number of sites meeting the data requirements for the trends analysis that
104 would allow meaningful analysis of patterns across the MARB. Moreover, formal state nutrient
105 loss reduction strategies started in the mid-2010's, which makes this period of particular interest
106 for the evaluation of implemented approaches.

107 3.1. Data harmonization and screening

108 The extensive TP concentrations reported in the Water Quality Portal for 30,369 sites within the
109 MARB by multiple local organizations, often with inconsistent methods, nomenclature and units,
110 were screened and harmonized to produce time series with consistent methods, units and
111 naming conventions. The TP dataset gathered all records of P mixed forms in unfiltered water
112 samples. Records from sites linked to the same stream segment were unified to increase the
113 length of the time series at these locations. This process was done through the COMID feature
114 of the National Hydrograph Dataset Plus (EPA, 2022).

115 The harmonization of the observed dataset plays a major role when analyzing historical
116 changes of nutrient loads (Sprague et al., 2017). Representatives and documentation by various
117 reporting agencies were consulted to clarify methods and units of ambiguous records. From TP
118 data, 23.2% and 16.4% of phosphate and phosphorus ambiguous records respectively, were
119 identified as P mixed forms through personal communication with agencies, while 28.7% and
120 19.4% of phosphate and phosphorus ambiguous records, respectively were resolved to P mixed
121 forms by analyzing reporting analytical methods. All P mixed forms records with ambiguous
122 reporting form (phosphate or phosphorus) were assumed to be in elemental form (Sprague et
123 al., 2017), and 31 out of 72 organizations reporting P mixed form records with ambiguous
124 fraction were resolved by inspecting all reported fractions for the specific organizations (Oelsner
125 et al., 2017). Harmonized concentration data were included in trend calculations only after
126 various screening criteria were met. This screening included the identification of duplicates,
127 censored, missing, zero, negative, outliers, and composite, control and field analyzed records.
128 The resolution of these records is reported in detail on (Botero-Acosta et al., 2025)
129 Subsequently, site screening removed sites that had more than 50% of left-censored data, less

130 than 70% quarterly coverage for the 2000-20 period, and less than 10% of decadal WQ data on
131 days with high flow regime (SF>85 percentile).

132 Streamflow (SF) sites located upstream or downstream of WQ sites were selected from the
133 USGS gage network when basin drainage areas had a maximum difference of 10% and no
134 dams were located in between the sites. Data of dams built before 2013 were extracted from
135 EPA (2022). SF data screening identified missing, zero and negative flow values. Missing
136 records were filled for years having no more than 3 consecutive and 30 total missing records
137 using the FillMiss function (USGS, 2016). All SF sites with longer nonconsecutive records for
138 the 1998-2022 water year periods were excluded.

139 3.2. Total Phosphorus trends

140 WRTDS (Hirsch et al., 2010) was used to calculate annual flow normalized (FN) loads and
141 concentrations. WRTDS has been widely used to characterize trends of nutrient loads (e.g.,
142 Oelsner et al., 2017; Stets et al., 2020; McIsaac et al., 2023) and to estimate the impacts of
143 management practices and flow on resulting trends (e.g., Murphy and Sprague, 2019). Here, we
144 used FN concentrations and loads assuming stationary and non-stationary flow to estimate the
145 total TP trends and the contribution of flow and all other factors to trends results (Botero-Acosta
146 et al., 2025). Analyses were conducted using the R statistical software program (R Core Team,
147 2017) and the EGRET R-package (Hirsch and De Cicco, 2015; Hirsch, 2018).

148 WRTDS concentration estimates were compared with observed values, and sites not having a
149 satisfactory performance were removed from the trends sites dataset. For this screening the
150 Pearson correlation coefficient, the extrapolation metric and the flux bias statistic were
151 estimated, and a visual inspection of residuals was performed to remove those sites with abrupt
152 residual changes, indicating a step change in the concentration that WRTDS would not be able

153 to accurately reproduce (Oelsner et al., 2017). Trends were then determined by the difference
154 between estimated FN concentration or load for 2020 and 2000. All TP concentration and load
155 (or yield) values referred to in the results section correspond to FN values. The FN values of the
156 obtained time series were used in the long-term trends estimation to reduce the impacts of
157 streamflow random variations on resulting nutrient time series (Hirsch et al., 2010; Murphy and
158 Sprague, 2019).

159 To enable the comparison of trends results among sites across the MARB, absolute changes
160 were normalized. Change in FN concentrations were normalized with respect to the value at the
161 beginning of the trend period (2000) and expressed as a percent. Annual changes in FN TP
162 load were normalized by drainage area, expressed as yield in kilograms per square kilometer,
163 were used in the analysis. Likelihood of trends was evaluated through a bootstrap test in which
164 a positive replicate fraction of 0.66 or larger would indicate a likely upward trend, 0.33 or smaller
165 would indicate a likely downward trend, and values between 0.33 and 0.66 would represent a no
166 likely trend (Hirsch et al., 2015; Yates et al., 2022).

167 The dominant trend component (streamflow vs non-streamflow) was defined as one that
168 exceeded the other component by a factor of 1.5 (absolute values). Watersheds where neither
169 component was dominant were classified as having mixed dominance. The percentage of
170 Influence of trends components (streamflow or non-streamflow) were computed as:

$$171 \quad \% \text{ Influence component } A = \frac{\text{abs}(\% \text{ change component } A)}{\text{abs}(\% \text{ change component } A) + \text{abs}(\% \text{ change component } B)} \quad \text{Eq.1}$$

172 3.3. Watershed features

173 Watershed features were gathered for drainage basins draining to the monitoring sites and
174 analyzed along with trends results similar to Botero-Acosta et al. (2025). Publicly available data

175 were consulted to extract basin area, land use, elevation, and upstream dam storage
 176 information. Dominant land uses for years 2001, 2011, and 2019 were identified from National
 177 Land Cover Dataset (NLCD) maps as the adapted land use category (Table 1) covering the
 178 largest area of the drainage basin (Stets et al., 2020). The dominant land use for the 2000-2020
 179 trend period was identified for those basins having the same land use category prevailing for the
 180 2001, 2011, and 2019 NLCD maps. When the dominant category changed during the 2000-
 181 2020 period, the label “Varied” was assigned. In addition, changes of percentage coverage from
 182 2001 to 2019 for each land use category were computed. A proxy for basin slope was included
 183 in our analysis: relief divided by drainage area (m/km²). Relief was extracted for basins draining
 184 to trends sites from a 5x5 degree DEM. Finally, dam storage information was extracted from the
 185 National Hydrography Dataset Plus (NHD-Plus) (EPA, 2022). We focused on readily available
 186 data that require a minimum of assumptions and estimation and are relatively stable. Future
 187 analyses will examine additional variables that are more dynamic and require various
 188 assumptions and approximation, such as fertilizer, manure, crop uptake and municipal
 189 wastewater.

190 **Table 1. NLCD categories grouped for watershed feature analysis.**

Adapted land use categories	NLCD Category ID#	Description
Cultivated (tilled land)	82	Cultivated Crops -areas used to produce annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20% of total vegetation. This class also includes all land being actively tilled.
Forest	41	Deciduous Forest
	42	Evergreen Forest
	43	Mixed Forest
Urban	22	Developed, Low Intensity
	23	Developed, Medium Intensity
	24	Developed High Intensity
Water	11, 12	Water
Wetlands	90, 95	Wetlands
Pasture/Hay	81	Pasture/Hay
Other	21	Developed, Open Space- areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses.
	31	Barren Land (Rock/Sand/Clay)
	52	Shrub/Scrub
	71	Herbaceous

191 Categorizing watersheds by dominant land cover could obscure the impacts of land use
192 changes that do not impact the dominant land cover. For instance, basins dominated by
193 cultivated land cover may have had relatively modest expansions of urban land cover that could
194 alter hydrology and nutrient loads but not change the dominant category as cultivated basins.
195 Hence, the percentage of individual land cover with respect to basin area for 2001 and the
196 change of this percentage during the trends period, were correlated with changes in TP
197 concentration and yield. Correlations among watershed features and between watershed
198 variables and water quality trend variables were analyzed through Pearson correlation (r), and
199 the statistical significance of correlations was evaluated by testing the null hypothesis of no
200 correlation with an alpha value of 0.05.

201 Finally, a cluster analysis was performed to identify similarities and discrepancies among
202 cultivated trend sites with likely increasing and decreasing TP yield trends. For this, the data of
203 predominantly cultivated sites was evaluated with the Ward's clustering method. The Ward's
204 method uses the incremental sum of squares technique to calculate the distance between all
205 objects in a cluster and its centroid and how this distance increase as clusters are merged
206 (MathWorks, 2024). The data used in the cluster analysis was normalized (with center 0 and
207 standard deviation 1) and scaled (from 0.15 to 0.85), to remove the impacts of scale differences
208 between variables and outliers (Patel and Mehta, 2011; Dalatu and Midi, 2020).

209 4. Results

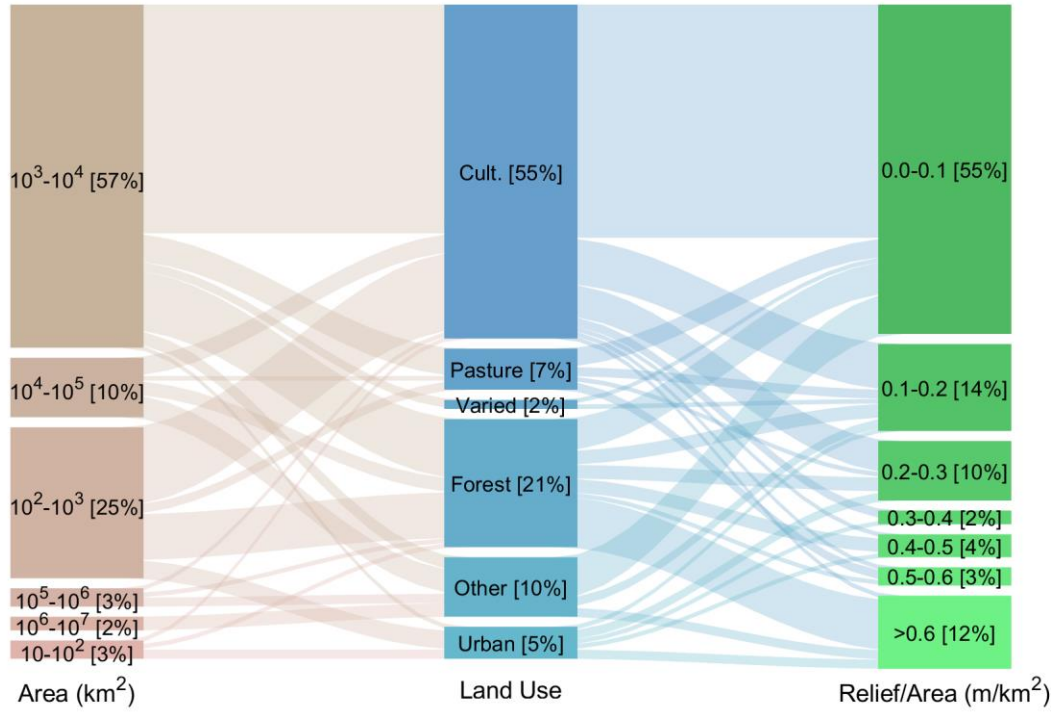
210 4.1. Watershed Features and TP Concentrations and Yields

211 Data harmonization and screening identified 132 sites that met the screening criteria and thus
212 were included in the trend analyses. Sites were distributed across the MARB but were

213 concentrated in the central portion of the basin (Figure S-1). Cultivated cropland was the
214 dominant landcover for 55% of the basins, followed by forested land (21%) (Figure 1, Table 2).
215 The dominant land covers remained unchanged during the 2000-20 period for all but two sites
216 and these were labeled as "Varied". Basin areas ranged from 70 km² to 17 x 10⁵ km², with 57%
217 of the sites ranging from 10³ to 10⁴ km². Relief per area had a minimum of 0.002 and a
218 maximum of 4.6 m/km², with 55% of sites having less than 0.1 m/km². Upstream dam storage
219 per drainage area ranged from 0 to 521,600 m³/km².

220 Predominantly urban basins had the largest mean FN concentration and yield, for both initial
221 and final years (Figure 2). Urban basins also had the largest FN yield ranges, while cultivated
222 basins had the largest ranges of FN concentration. By the final year, maximum and mean
223 values from urban dominated sites had declined, while the values from cultivated dominated
224 basins had remained the same or increased. Watersheds with pasture as the dominant land
225 cover tended to have larger mean and maximum FN yields than cultivated, forest, and varied
226 dominated sites, but generally lower than those dominated by urban land cover.

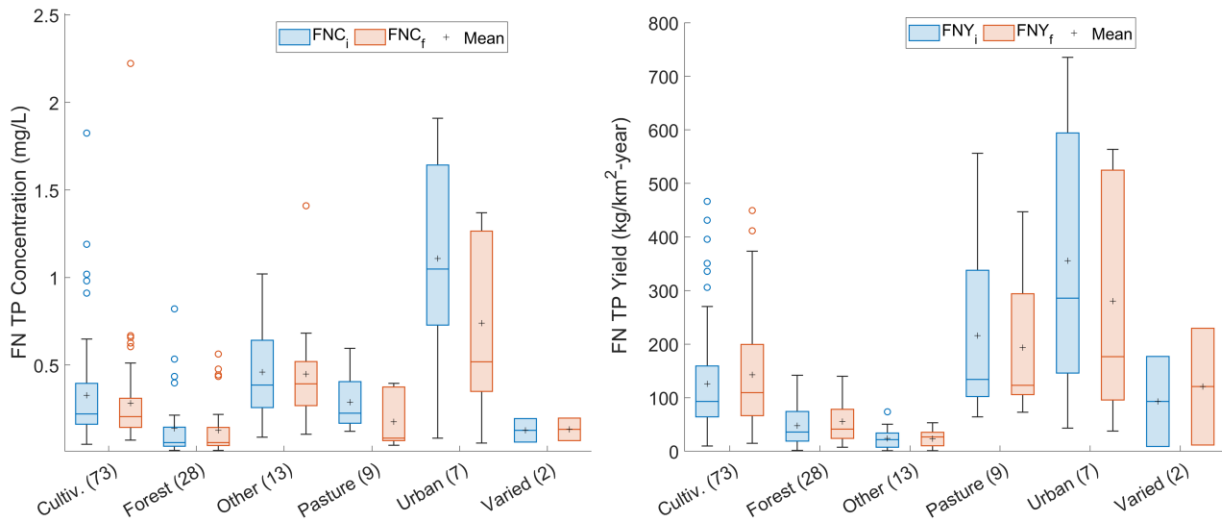
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Figure 1. Main watershed features for the 132 total phosphorus (TP) trends sites and percentage of sites for each category.

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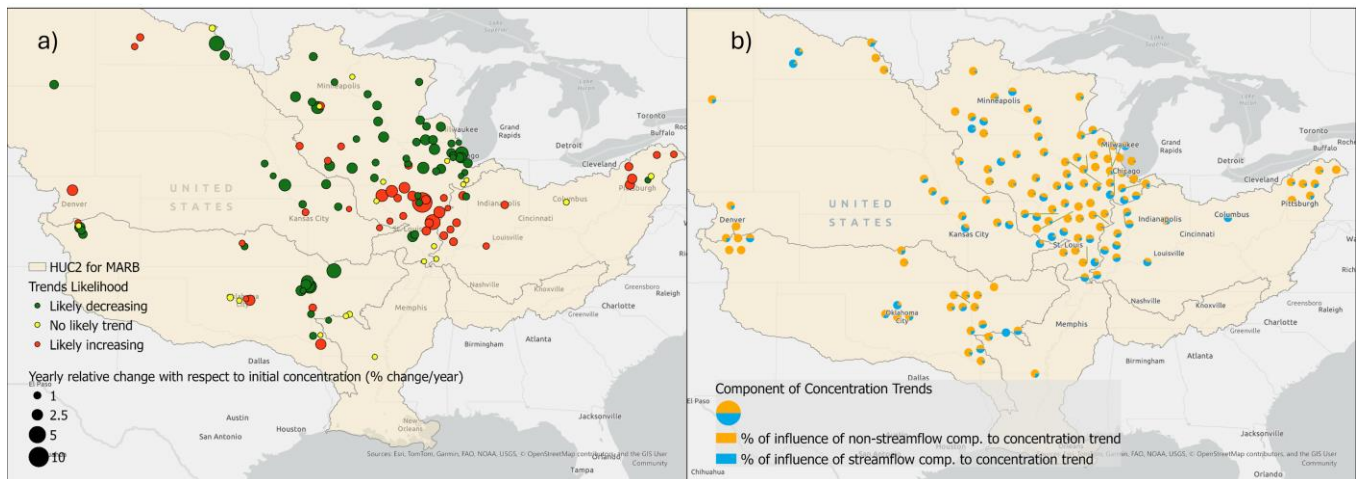
Figure 2. Box plots of flow normalized (FN) concentration and yield at the initial (i) and final (f) years of trends period per dominated land cover.

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234 4.2. Overall trends

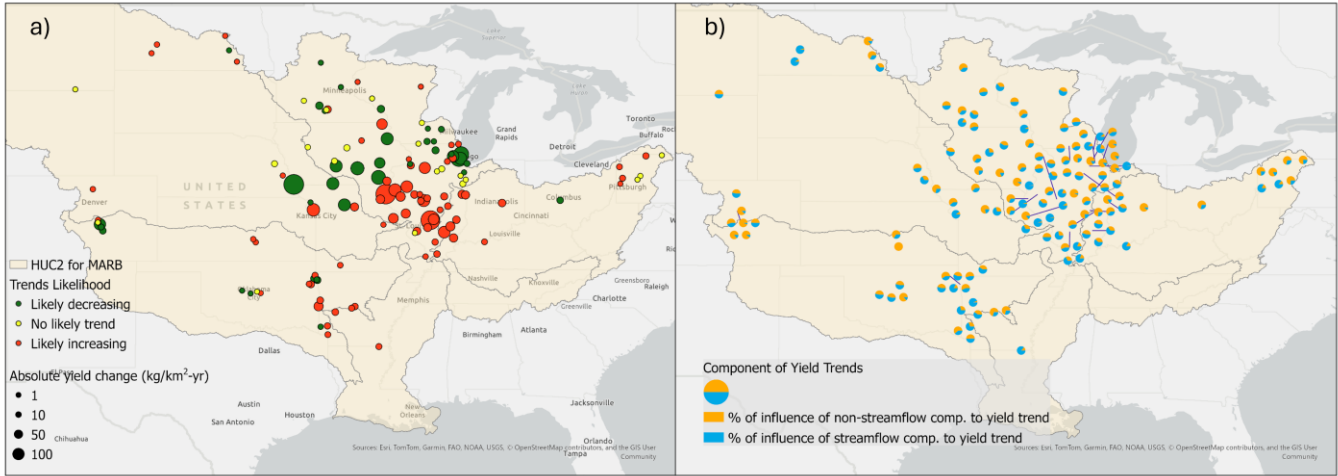
235 The percentage of sites with likely decreases of FN concentration (51%) exceeded the 33% of
236 sites with likely increases (Table 2). For yield trends, the percentages of decreasing and
237 increasing were approximately reversed with 29% of sites having likely decreasing and 55% of
238 sites likely increasing trends. There is some geographic clustering of trend results, with several
239 increasing FN concentration and FN yield trends in the central portion of the basin, and a cluster
240 of decreasing trends in the northern portion of the basin (Figure 3 and Figure 4).



241
242 **Figure 3. a) TP concentration trends for the 2000-2020 period (n=132). Size of circles were**
243 **set based on annual percentage change; colors were set based on trends likely direction.**
244 **b) Percent influence of streamflow and non-streamflow components.**

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Figure 4. a) TP yield trends for the 2000-2020 period (n=132). Size of circles were set based on absolute magnitude of total trends; colors were set based on trends likely direction. b) Percent influence of streamflow and non-streamflow components.

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Table 2. Distribution of sites by likely TP trends and dominant land cover.

Dominant Land use	% of sites	Total area (km ²)	% of sites for concentration trend categories			% of sites for yield trend categories		
			Likely Increasing	Likely Decreasing	No likely Trend	Likely Increasing	Likely Decreasing	No likely Trend
Varied (2)	1.5%	4,814	50.0%	0.0%	50.0%	100.0%	0.0%	0.0%
Other (13)	9.8%	4,581,660	38.5%	38.5%	23.1%	46.2%	38.5%	15.4%
Pasture (9)	6.8%	33,463	11.1%	88.9%	0.0%	44.4%	55.6%	0.0%
Urban (7)	5.3%	2,822	0.0%	100.0%	0.0%	14.3%	85.7%	0.0%
Forest (28)	21.2%	356,996	32.1%	35.7%	32.1%	64.3%	10.7%	25.0%
Cultiv. (73)	55.3%	476,970	38.4%	50.7%	11.0%	56.2%	26.0%	17.8%
MARB (132)	100.0%	5,456,724*	33.3%	50.8%	15.9%	54.5%	28.8%	16.7%

*Total drainage area is larger than the MARB because there are overlapping drainage areas in basins with multiple monitoring locations. For instance, the drainage area of the Mississippi River at Thebes includes all the upstream basins, including the Missouri River at Herman

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For the 73 basins dominated by cultivated cropland, the percentages of sites with likely

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increasing, decreasing and no trends are similar to the percentages for all 132 basins across

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the MARB as described above. For the 28 basins dominated by forest land cover, FN

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concentration trends were nearly equally divided among increasing, decreasing and no likely

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trend; but for FN yield, 64.3% of the forest dominated basins had likely increasing trends. For

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the nine basins dominated by pasture, 88.9% had likely decreasing FN concentration trends; but

258 for FN yields, pasture dominated basins were nearly equally divided between likely increasing
 259 and decreasing trends. For the seven basins dominated by urban land cover, all had likely
 260 decreasing FN concentration trends, and all but one had likely decreasing FN yield trends.

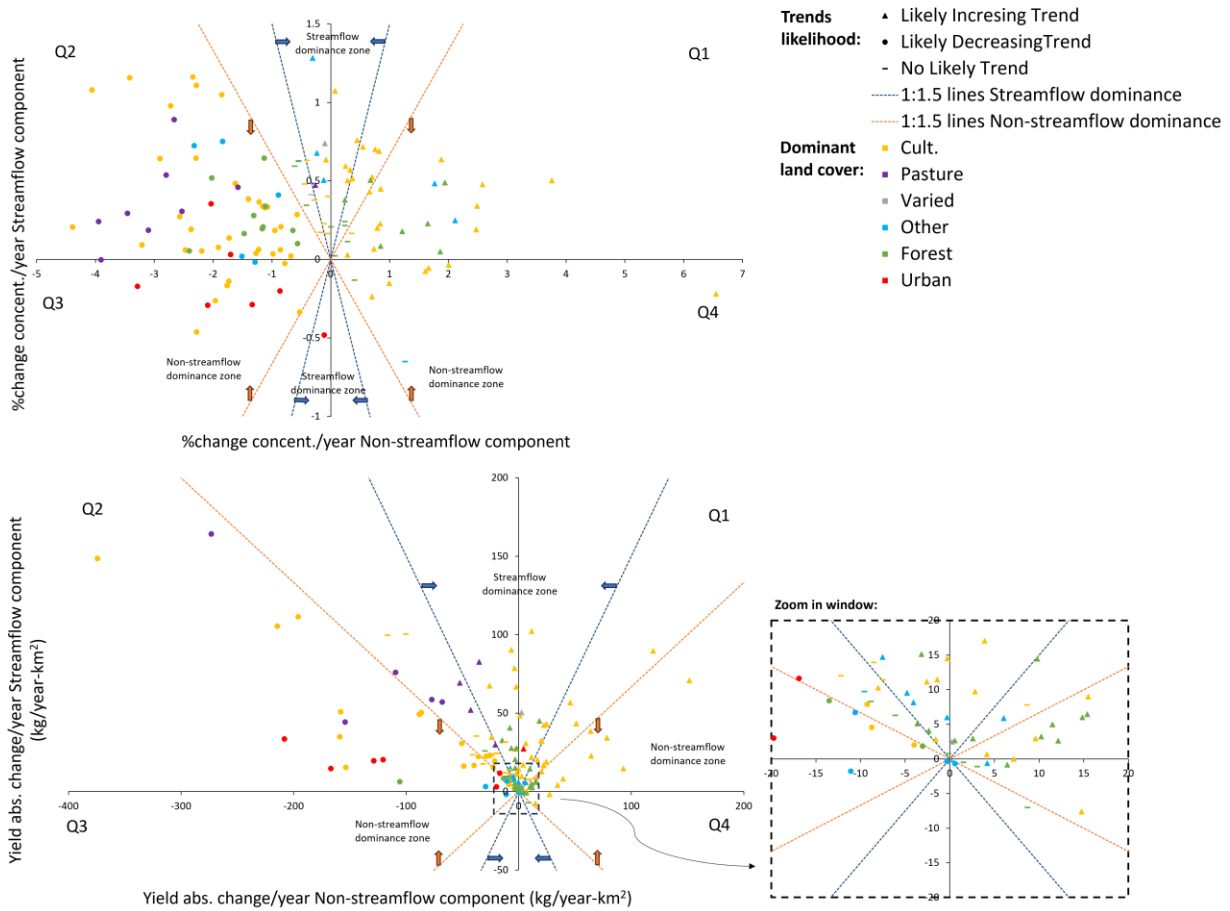
261 **4.3. Trend Components**

262 Likely changes of FN TP concentration during the 2000-2020 period were more frequently
 263 dominated by the non-streamflow component (70% of sites), with 20% of sites likely increasing
 264 and 50% of sites decreasing. In contrast, FN TP yield likely changes were more evenly
 265 distributed among sites with non-streamflow dominance (36% of sites), streamflow dominance
 266 (27% of sites), and mixed dominance (20% of sites). With the non-streamflow dominated sites
 267 having both likely yield increases (16%) and decreases (20%), and the streamflow dominated
 268 sites having only significant yield increases (27% of sites) (Figure 5, Figure S-2, Figure S-3).
 269 Total FN yield trends were more strongly and positively related to the non-streamflow
 270 component for sites with decreasing trends ($R^2= 0.85$) than sites with increasing trends
 271 ($R^2=0.65$) (Figure S-4). The streamflow component was positively related to increasing total FN
 272 TP yields ($R^2=0.47$), but weakly ($R^2= 0.21$) and negatively related to decreasing total yield
 273 trends.

274 **Table 3. Mean, maximum and minimum FN TP trends for dominant land uses. NS= Non-**
 275 **streamflow component**

	% change/year Total trend concent. (%/year)			% change/year NS trend comp. concent (%/year)			% change/year SF trend comp. concent. (%/year)			abs. change/year Total trend Yield (kg/km ² -yr)			abs. change/year NS trend comp. Yield (kg/km ² -yr)			abs. change/year SF trend comp. Yield (kg/km ² -yr)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Varied (2)	0.36	0.64	0.08	-0.22	-0.10	-0.34	0.58	0.74	0.41	28	53	3	1	2	0	26	50	2
Other (13)	0.10	2.36	-1.60	-0.29	2.11	-2.32	0.39	1.28	-0.65	-1	12	-26	-5	6	-29	5	15	-2
Pasture (9)	-2.32	0.21	-3.91	-2.69	-0.26	-3.95	0.38	0.89	0.00	-22	47	-110	-93	-21	-273	70	164	30
Urban (7)	-1.78	-0.59	-3.46	-1.63	-0.11	-3.28	-0.15	0.35	-0.48	-75	32	-175	-94	4	-208	19	34	3
Forest (28)	0.11	2.43	-2.35	-0.13	1.94	-2.41	0.24	0.64	-0.13	8	63	-99	-3	18	-106	11	45	-7
Cultiv. (73)	-0.13	6.33	-4.18	-0.45	6.55	-4.39	0.32	1.16	-0.46	17	222	-226	-16	152	-374	33	149	-8
MARB (132)	-0.28	6.33	-4.18	-0.58	6.55	-4.39	0.29	1.28	-0.65	6	222	-226	-22	152	-374	27	164	-8

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279 **Figure 5. Non-streamflow component vs Streamflow component for TP concentration and**
 280 **yield trends. Markers and colors represent the trend likelihood and the dominant land**
 281 **use category, respectively.**

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283 **Table 4. Percentage of sites per trend likelihood and quadrant in Figure 5.**

	<i>Concentration trend categories (% of sites)</i>				<i>Yield trend categories (% of sites)</i>			
	Likely Increasing	Likely decreasing	No Likely Trend	Cumulative Quadrant	Likely Increasing	Likely decreasing	No Likely Trend	Cumulative Quadrant
Q1	22.73%	0.00%	3.79%	26.52%	31.06%	0.00%	0.76%	31.82%
Q2	4.55%	40.15%	9.85%	54.55%	20.45%	26.52%	12.88%	59.85%
Q3	0.00%	10.61%	0.00%	10.61%	0.00%	1.52%	0.00%	1.52%
Q4	6.06%	0.00%	2.27%	8.33%	3.03%	0.76%	3.03%	6.82%

284

285 Figure 5 illustrates the TP trends likelihood, the dominant land cover, and the magnitude of each
286 trends component for all TP trends sites. The graph domain can be divided using two methods,
287 by dominance zones of streamflow and non-streamflow components and by quadrants. The
288 former indicates the relative importance of each trends component with respect to the total
289 absolute change observed in the FN TP variable during the 2000-2020 period at the site, and
290 the latter splits the sites into 4 regions (quadrants) with increases or decreases of each trends
291 component. Quadrant 1 (Q1) indicates sites with increases in both streamflow and non-
292 streamflow components, while quadrant 3 (Q) indicates decreases in both components.
293 Quadrant 2 (Q2) and 4 (Q4) represent increases in one component and decreases in the other
294 component. Of the sites in Q1, nearly all had increased TP yield except one site had no trend
295 (Table 4). Most sites were in Q2 in which the vast majority had decreasing concentration trends
296 and a slight plurality had decreasing yield trends. Relatively few sites were in Q3 or Q4.

297 4.4. Correlation Analysis

298 Correlation analysis identified strong positive correlations between the non-streamflow
299 component and the total trend for both yield ($r=0.89$) and concentration ($r=0.98$) changes of
300 riverine TP, as well as positive correlation between total concentration and yield trends ($r=0.57$)
301 (Figure S-5). The percentage of urban land was positively correlated to 2000-values of TP yield
302 ($r=0.50$) and concentration ($r=0.58$) and was also slightly negatively correlated to changes in TP
303 yield ($r=-0.36$) and streamflow trend component of concentration ($r=-0.38$). The percentage
304 coverage of pastures had a positive correlation with streamflow component of TP yield trend
305 ($r=0.46$) but a slightly negative correlation ($r=-0.21$) with the non-streamflow component and no
306 correlation with total TP yield trend.

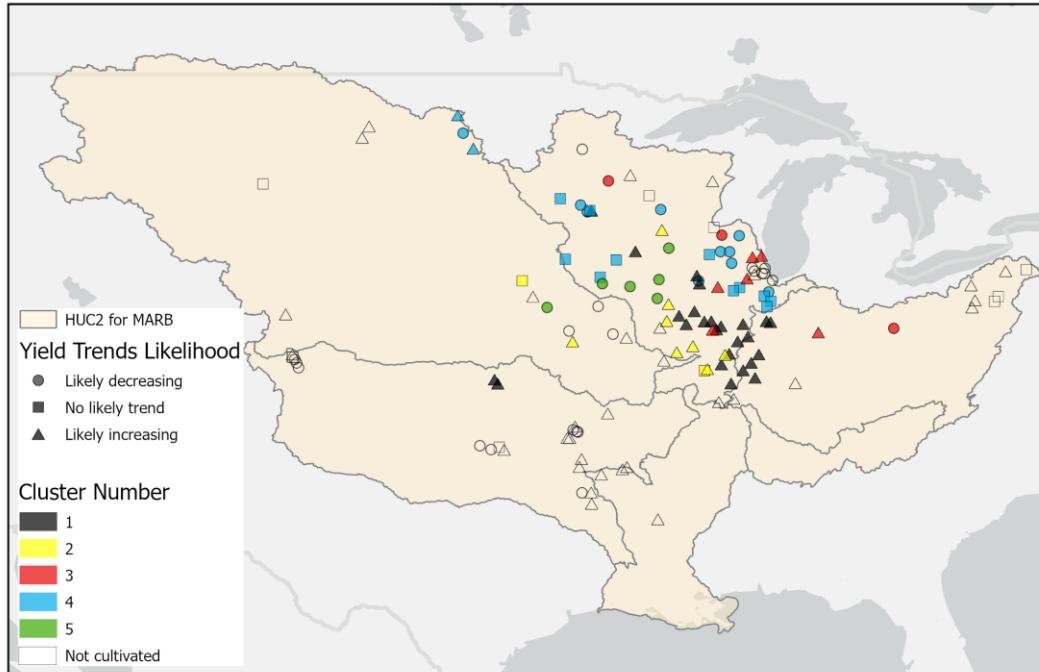
307 Land cover changes based on NLCD for 2001 and 2019 revealed that the greatest increases in
308 basin area coverage for individual sites were observed for Other (14.9%) and Urban (10.2%)

309 categories (described in Table 1). The maximum decreases were 13.6% followed by 12.8% of
310 basin area for the Forest and Cultivated categories, respectively. The correlation analysis
311 between TP concentration and yield trends, and land cover changes (Figure S-6) provided a
312 strong negative relationship between changes in pasture coverage and changes on the
313 streamflow trend component of TP yields ($r = -0.54$), which might be related to the slight positive
314 correlation between the latter and changes in cultivated land ($r = 0.35$) to the extent that these
315 land cover changes involved conversion of pasture to cropland.

316 4.1. Cluster analysis for cultivated basins

317 The Ward's clustering analysis for the FN yield dataset composed of sites with predominantly
318 cultivated basins identified five clear clusters, denoted with the numbers 1 to 5 (Figure S-8),
319 from the variables listed in Figure S-7. Sites belonging to each cluster and yield trend likelihood
320 were plotted in Figure 6. Results evidenced that the largest likely decreases of FN yield (green
321 clusters, mean total TP trend $-131.4 \text{ kg/km}^2\text{-yr}$) occurred at sites located towards the central
322 region with large initial yield values and large reductions in the NS trend component. The largest
323 likely increases (yellow cluster, mean total TP trend $84.2 \text{ kg/km}^2\text{-yr}$) had the combined impact of
324 large SF and NS yield trends components and occurred in the southern region at basins having
325 medium initial FN yields. Smaller likely increases were observed for the black cluster (mean
326 total TP trend $45.5 \text{ kg/km}^2\text{-yr}$), also located towards the south, having smaller increases on the
327 SF and NS components, and smaller initial yield values with respect to the yellow cluster.
328 Finally, the red cluster (mean total TP trend $18.5 \text{ kg/km}^2\text{-yr}$), grouped all sites with large slopes
329 and small initial yield values, mostly having small likely changes of TP yield

330



331

332 **Figure 6. Yield trends likelihood and location of sites belonging to identified clusters**
 333 **(denoted 1-5) for predominantly cultivated basins.**

334 **5. Discussion**

335 Our results indicated that watersheds dominated by urban, pasture, and cultivated land covers
 336 had the largest FN TP yields in 2000 and 2020 (Figure 2). These land cover categories typically
 337 include main sources of P in the MARB: sewage effluents from urban areas, fertilizer application
 338 on cultivated land, and manure losses from unconfined animals in pastures/rangeland . While a
 339 majority of FN yields from urban and pasture dominated basins tended to decrease during the
 340 2000-20 period, a majority of TP yields from cultivated basins tended to increase (Table 2). A
 341 slim majority of cultivated basins had likely decreasing concentration trends, while nearly all the
 342 urban and nearly all the pasture dominated basins had likely decreasing concentration trends
 343 during our analyzed period. The percentage of sites with likely increases and decreases during
 344 the 2000-20 period inverted for concentration and yield variables, with more sites having likely

345 increases of FN yield (55% of sites) than FN concentrations (33% of sites). For both FN
346 variables a cluster of increases was observed in the central region of the basin. Our results
347 contrast with those of Sprague and Lorenz (2009), who reported a historical significant regional
348 increasing trends of flow adjusted concentration values in the central region of the continental
349 U.S. (which contains most of MARB area) significantly correlated to inputs from fertilizers.
350 According to Sprague and Lorenz (2009) there were more increases than decreases of flow-
351 adjusted TP concentration values in this region during the 1993-2003 period, while our results
352 show that more sites had likely decreases than increases of FN concentration throughout the
353 MARB. Reasons for this discrepancy could be changes in practices from the 1993-2003 period
354 to the 2000-2020 period, basin characteristics of the analyzed trend sites, and methodological
355 differences as their concentration values were not subjected to the WRTDS flow normalization.

356 Sites simultaneously having likely decreases in concentration and likely increases in yield
357 corresponded to 16% of the sites, almost all having streamflow or mixed dominance for the yield
358 trends, and associated with predominantly Pasture, Forest, and Cultivated basins. These results
359 indicated the larger role of streamflow component on riverine TP yield increases. These results
360 are in some ways similar to those reported for 110 sites across the continental USA for the 1982
361 to 2012 period in which likely FN TP concentration trends tended to be dominated by the non-
362 streamflow component while yield trends had relatively more mixed and streamflow dominance
363 component (Murphy and Sprague, 2019). However, Murphy and Sprague reported only 5% of
364 sites with streamflow dominance in load trends compared to our value of 27%. This difference
365 may be due to differences in sites and time periods analyzed.

366 Sites with likely increases for both concentration and yield (39 sites), were predominantly
367 cultivated basins located at the south portion of the Upper Mississippi Region (central region of
368 the MARB) (Figure 3 and Figure 4). Although streamflow had a major influence on these sites,

369 other factors were also influential. In most of these sites both concentration and yield were
370 dominated by the same component (10 sites by streamflow component and 16 sites by non-
371 streamflow).

372 The percentage of sites having likely increasing or decreasing trends in each dominance zone
373 and quadrant of Figure 5 and Table 4 conveys the relative importance of streamflow vs. non-
374 streamflow components on resulting trends. Q2 is of special interest given the extensive
375 literature in which increased annual flows correlate with increased annual TP loads at a site
376 (Gentry et al., 2007; Basu et al., 2010; Jarvie et al., 2012; Baker et al., 2014; Dolph et al., 2019;
377 Rowland et al., 2021). Our results show that more than half of the sites (54.5% and 59.8% for
378 concentration and yield trends sites, respectively) are located in Q2, for which the majority for
379 concentration (73.6% of sites in Q2) and close to half of them for yield (44.3% of sites in Q2)
380 experienced likely decreasing total trends. The separation of trends likelihood inside Q2 was
381 highly related to the component dominance zones, with all increases of TP concentration inside
382 the flow dominance zone, and all increases in TP yield in the flow dominance or mixed-
383 dominance zones. In our trends dataset, none of the 40 sites with streamflow dominance on
384 yield trends, and only 1 of the 18 sites with streamflow dominance on concentration trends
385 experienced decreases, regardless of other characteristics (Figure 5, Figure S-2, Figure S-3). In
386 other words, all but one of the 67 sites with likely decreases in concentration had non-
387 streamflow component dominance.

388 Focusing on a few sites with the largest magnitude trends reveals some site-specific factors that
389 can be influential yet difficult to generalize across the MARB. A site with by far the largest
390 increase in TP total concentration trend for the 2000-20 period was in the Cultivated category
391 with a maximum annual concentration increase of 6.3%/yr with respect to 2000 values (Table 3).
392 This site (Sangamon River at Riverton, IL) had 96.76% influence of the non-streamflow

393 component for the concentration trends and was impacted by unusually large increases in
394 wastewater P discharge from Decatur (McIsaac et al., 2023). The maximum annual yield
395 increase of 222.4 kg/km² was calculated for Bear Creek at Marcelline, IL, a site draining
396 cultivated land with little urban land cover, relief per area of 0.094 m/km² and three upstream
397 dams (with a storage of 1.37x10⁶ m³). This location has 68.2% of non-streamflow component
398 influence on yield trends (Figure 4). The second largest annual FN TP yield increase (209
399 kg/km²) was observed at Hurricane Creek (Illinois), a cultivated basin with a relief per area of
400 0.229 m/km² and one upstream dam (1.28x10⁵ m³). This site has experienced large flow
401 increases during the 2000-2020 period, which were evidenced in the importance of both trend
402 components (42.9% for streamflow and 57.1% for non-streamflow). These three sites are part of
403 the cluster of sites with increased concentrations and yields in the central part of the MARB. The
404 variation in conditions among the sites illustrate some of the difficulty of identifying a common
405 cause for the geographic cluster of sites with increased concentrations and yields.

406 Sites with the largest decreases in TP concentration belonged to Cultivated and Pasture
407 categories with maximum annual decreases of 4.2%/yr and 3.9%/yr with respect to 2000 values,
408 respectively, these sites were located in North Dakota and Missouri (Figure 4). The largest yield
409 decrease (226 kg/km²) was observed in Nishnabotna River, IA, draining largely cultivated land
410 with a 0.032 m/km² relief per area Figure 4. This site had 71.6% influence of non-streamflow
411 component on yield trends, indicating its dominance over the flow component. Average annual
412 flow at this site doubled from early 2000 to late 2010. These abrupt variations might have been
413 treated as random variations and underestimated during the flow normalization process, which
414 produced larger FN yields for 2000, that led to the reported reduction. However, a historical
415 analysis of streamflow records at this site (1930-2020) showed a clear increasing long-term
416 tendency. To overcome this issue, a longer time series of nutrient concentration (>30 years)

417 would be recommended to better capture the long-term impacts of flow in riverine nutrient
418 trends. This will be possible if sites maintain sampling in the upcoming decades.

419 Correlation results and analysis by dominant land cover indicated basins with large urban areas
420 tended to have large yields of TP at the beginning of trend period and tended to decrease. All
421 urban basins (7) but one experienced likely decreases of concentration and yield, with non-
422 streamflow being the dominant yield and concentration trend component. The only urban basin
423 having likely decreases for concentration with likely increases of yield had flow dominance for
424 both yield and concentration trends. Reductions of TP concentrations in U.S. urban basins have
425 been previously reported for the 1992-2012 period by Stets et al. (2020). These reductions may
426 be explained by improved wastewater treatment, and an increase in the magnitude and
427 frequency of extreme rainfall events, producing more runoff without fertilizer inputs from
428 cultivated land, causing a diluting impact on concentrations.

429 Although, TP yield values have been positively correlated to catchment area and basin slope
430 (e.g.,Allafta et al., 2021), our results did not evidence significant correlations of riverine TP
431 changes with neither of these variables (slope analyzed through the relief/area variable). This
432 could be explained by the correlations of basin area and slope with the percentage of area for
433 each land cover. For our dataset, slope (represented by relief/area) was significant and strongly
434 positively correlated ($r=0.58$) with percent of forest land and significant and negatively correlated
435 ($r=-0.45$) with percent area of cultivated land. This indicated that the steeper the basin, the more
436 forested coverage and the less cultivated land, similarly to what occurred in Jacobson et al.
437 (2011). Cultivated or urban land covers are related to TP yield increases (Sharpley and Smith,
438 1989; Lou et al., 2015; Allafta et al., 2021), while forested land has been related with low TP
439 values due to its limited P inputs and dense vegetation cover that intercepts raindrops and
440 reduces erosion (Zhuang et al., 2015; Kim et al., 2018; Allafta et al., 2021). On the other hand,

441 basin area was found to be significant and slightly correlated ($r=0.35$) with percent of Other land
442 cover, which represents barren and shrub covered land, not having significant P inputs.

443 When we focused the analysis on the yield trends of predominantly cultivated sites, the non-
444 streamflow component had the largest impact on likely decreases of yields (Figure S-7) while
445 the majority of sites with yield increases were dominated by the streamflow component or had
446 mixed dominance (Figure S-9). Cluster results showed a large group of sites with likely
447 increasing trends associated with cultivated basins located at the south-central region (black
448 and yellow clusters), with the black cluster having smaller initial FN yields and SF trend
449 components compared to the yellow cluster. Plotting the changes in the percentage of basin
450 area with cultivated and urban land during the trends period for each cluster (Figure S-10),
451 evidenced that sites in the yellow cluster tended to experience a larger expansion of cultivated
452 land during the analysis period compared to the black cluster. Although also experiencing
453 cultivated land expansion, the green cluster, located in the central region had the largest
454 decreases. This may possibly be explained by regional differences in the implemented nutrient
455 management practices. Overall results showed a trend toward FN TP yield increases at lower
456 latitudes.

457 5.1. Uncertainties and limitations

458 Flow normalized concentrations and yields are estimates of values that are hypothetically
459 expected to occur under “normal” flow conditions based on past statistical probabilities, not on
460 mechanistic modeling of the interactions of concentration and flow. Since flows are rarely
461 “normal” (however normal is defined), FN concentrations and loads may differ substantially from
462 actual concentrations and loads in a given year, which are causes of eutrophication and are the
463 targets of nutrient reduction efforts. Flow normalization can be useful in estimating relative

464 impacts of changes in hydrology versus other factors, these estimates should be understood to
465 be a mean value when integrated over the frequency distribution of discharge.

466 Trend period selection can impact the trends magnitude and direction, since trends are
467 estimated as the difference in concentration and yield between initial and final years. The
468 identification of long-term flow variations and their impacts in the trends results might be
469 impacted if the trends period is not long enough to discern random variations from non-
470 stationary flow variability, especially at sites with extreme flow events.

471 Analysis of factors influencing riverine nutrient trends is limited by available data and resources.
472 Data such as fertilizer and manure applications and crop nutrient uptake are undergoing a
473 process of updating and refinement that is expected to be completed in 2025. These updated
474 data should be incorporated into future studies of factors influencing riverine nutrient trends.

475 6. Conclusions

476 Of the 132 sites in the MARB that met our data screening criteria for TP trend analyses, 33%
477 had likely increasing FN concentration trends, 51% had decreasing FN concentration trends and
478 the remaining had no likely trend between 2000 and 2020. Fewer sites (29%) had likely
479 decreasing FN yield trends, including six of seven basins dominated by urban landcover. Likely
480 increasing yield trends occurred at 55% of sites and the remaining 16% had no likely yield trend.
481 The streamflow component of FN concentrations and yields were greater than zero for 81% and
482 92% of the sites, respectively (Q1 and Q2 in Figure 5), indicating increases in streamflow were
483 widespread across the MARB. Nearly all of the 55% of the sites with likely increasing TP yield
484 trends had streamflow components greater than zero and were somewhat evenly distributed
485 between positive and negative non-streamflow components. Nearly all of the 29% of sites
486 having decreasing TP yield trends had streamflow components greater than zero and negative

487 non-streamflow components, indicating that TP load reductions occurred even where long-term
488 streamflow trends increased.

489 Long-term changes of streamflow were the dominant component of likely trends in FN TP
490 concentration and yields for only 10% and 27% of the sites, respectively. All other factors, herein
491 referred to as non-streamflow component, were the dominant component for 70% and 36% of
492 the sites for concentration and yield trends, respectively. Among sites with non-streamflow trend
493 dominance, likely decreases of TP concentration were more common than increases, while
494 likely increases and decreases of TP yields were both observed with similar frequency. The non-
495 streamflow component was strongly correlated ($r>0.89$) with changes (total trends) in FN TP
496 concentrations and yields. This non-streamflow component includes all factors other than flow
497 variation, such as fertilizer, manure, point source discharges, stream channel erosion, legacy
498 nutrients and sinks, none of which were quantified in our analysis, and warrant further detailed
499 investigation.

500 Statistical analyses of patterns and trends across the MARB as related to basic watershed
501 characteristics resulted in weak correlations with limited generalizability. Thus, better
502 understanding of factors influencing temporal and spatial variation in TP concentration and yield
503 trends requires consideration of a wider range of factors (e.g., nutrient budgets) and/or regionally
504 relevant factors (e.g., climate, soils, tile drainage).

505 7. Data availability

506 Sites information and detailed results of FN concentration and loads will be made available at
507 the Great Lakes to Gulf Virtual Observatory web site ([https://greatlaketogulf.org/nutrient-](https://greatlaketogulf.org/nutrient-trends)
508 [trends](https://greatlaketogulf.org/nutrient-trends)).

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