

A Synoptic Survey of Nitrogen and Phosphorus in Tributary Streams and Great Rivers of the Upper Mississippi, Missouri, and Ohio River Basins

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Abstract We combined stream chemistry and hydrology data from surveys of 436 tributary stream sites and 447 great river sites in the Upper Mississippi, Missouri and Ohio River basins to provide a regional snapshot of baseflow total nitrogen (TN) and total phosphorus (TP) concentrations, and to investigate the relationships between land use and stream chemistry. Catchments in the Upper Mississippi River basin had more land in agricultural uses (51%) than the Missouri or Ohio River basin catchments (25% and 29%, respectively). The difference in agriculture is reflected in the TN concentrations in tributary streams and the great rivers: 5,431 and 2,112 $\mu\text{g L}^{-1}$ for the Upper Mississippi, 1,751 and 978 $\mu\text{g L}^{-1}$ for the Missouri, and 1,074 and 1,152 $\mu\text{g L}^{-1}$ for the Ohio River basins. This agricultural effect was not as evident for tributary stream or great river TP concentrations: 165

and 181 $\mu\text{g L}^{-1}$ in the Upper Mississippi, 177 and 171 $\mu\text{g L}^{-1}$ in the Missouri, and 67 and 53 $\mu\text{g L}^{-1}$ in the Ohio River basins. We set reference thresholds based on the 75th percentile TN and TP concentrations at our least disturbed sites. The TN threshold was exceeded for 50–63% of the tributary stream and 16–55% of great river lengths, with the greatest proportion in the Upper Mississippi River basin. The TP threshold was exceeded in 32–48% of tributary stream and 12–41% of great river lengths. Tributary stream N/P ranged from 67:1 (Ohio) to 210:1 (Upper Mississippi); river N/P ranged from 20:1 (Missouri) to 60:1 (Ohio). N/P indicated that potential N-limitation occurred in 10–21% of total tributary stream length and in 0–46% of great river length; potential P-limitation ranged from 60–83% of cumulative tributary stream length and from 21–98% of cumulative great river length. Total N flux (concentration \times discharge) was highest in the Upper Mississippi River basin; TP flux was lowest in the Ohio River basin. River TN yields and TP yields for both tributary streams and great rivers, was not significantly different between the sub-basins. Our study empirically links catchment land use and stream chemistry, and demonstrates using monitoring data for estimating nutrient yields at a large regional scale.

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Spatial analysis

1 Introduction

Stream ecosystems around the world are impacted by the development of their catchment for human uses. These developments, including agriculture, urbanization, industry, and the associated increases in municipal effluents and channel modifications, are occurring at unprecedented rates resulting in increased alteration, impairment, and destruction of stream ecosystems (Broussard and Turner 2009; Palmer 2009). Nutrient and sediment influx to streams as nonpoint source pollution is the leading cause of water quality degradation in streams of the United States (Turner and Rabalais 2003; Broussard and Turner 2009).

The United States Clean Water Act (CWA) was enacted in 1972 to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters." The CWA has resulted in improved water quality through the reduction of point sources of contaminants, but has failed to curb habitat loss and nonpoint sources of nutrients and sediments (Adler et al. 1993). Examples of this nonpoint source pollution abound (Goolsby et al. 1999; McIsaac et al. 2002; Dodds 2006). Most states in the Mississippi River basin have many miles of river impaired by high nutrient concentrations and are not fully supporting their designated aquatic life uses (Turner and Rabalais 2003; US Environmental Protection Agency 2007a). One problem in particular, the relationship between nutrients and the extent of the Gulf of Mexico hypoxic zone, has risen to national prominence (Rabalais et al. 2002; Turner and Rabalais 2003, 2004; Dodds 2006). Since 1985, the Northern Gulf of Mexico hypoxic zone has ranged from about 8,000 to about 22,000 km². This area, where the summertime bottom water dissolved oxygen concentrations fall below 2 mg O₂ L⁻¹, has been causally linked to excessive nutrients, primarily nitrogen (N), exported to the Gulf of Mexico from the Mississippi River basin (MRB, Turner and Rabalais 2003; Dodds 2006). Nitrogen export to the Gulf of Mexico has tripled since the 1950s, and is attributed to a 30-fold increase in N fertilizer application, increased tile drainage, and increased discharges in the MRB (Turner and Rabalais 1991; Mitsch et al. 2001; Rabalais et al. 2002; Donner et al. 2002, 2004; Broussard and Turner 2009).

Regional scale assessment of stream water quality requires a monitoring design that allows accurate extrapolation of site data to the entire population of stream resources in question (Hawkins et al. 2008). Survey design approaches that have been used for regional scale stream monitoring include the snapshot methodology (Grayson et al. 1997; Salvia et al. 1999) and probability-based surveys (Olsen and Peck 2008; Angradi et al. 2009). The snapshot methodology samples a river network at every confluence within a river basin during a period of stable, low flow. This approach allows relative load estimations as a function of flow accumulation, catchment geology, and land use with minimal influence of climate. By comparison, the probability survey approach used by the USEPA also samples streams during low flow conditions, though over a much longer time period (Olsen and Peck 2008; Angradi et al. 2009). The probability surveys differ from the snapshot technique in that the sites are randomly selected and not targeted at river confluences. The strengths of the snapshot approach are its abilities to apportion load among point source and nonpoint sources and to identify reaches that have excessive loads or higher retention capabilities (Grayson et al. 1997). The strength of the probability survey is its ability to infer condition at large spatial scales from a relatively small number of randomly selected sites.

The snapshot methodology has been applied to river basins up to 5,000 km² (Grayson et al., 1997). In contrast, Olsen and Peck (2008) describe a probability design for reporting on wadeable stream conditions for all perennial streams of the conterminous USA, and Angradi et al. (2009) present a survey of conditions of the great rivers of the Missouri, Upper Mississippi, and Ohio River basins. Here, we combine data from these two probability designs to report stable, low flow nutrient concentrations in, and fluxes from these three basins, a combined area of 1.8×10^6 km². We pair chemistry and hydrology data with catchment area and land use information to investigate the relationship between nutrient concentrations, fluxes, and yields across the Upper Mississippi, Missouri, and Ohio River basins. Lastly, we set reference expectations for the tributary streams and great rivers within these basins, and assess the proportions of their total lengths exceeding those thresholds.

2 Methods

2.1 Site Selection

Tributary stream sites included in this study were the 436 first through eighth-order (Strahler 1957) streams in the Upper Mississippi (82), Missouri (238), and Ohio (116) River basins that were sampled during the US Environmental Protection Agency's assessment of wadeable streams of the conterminous USA (the Wadeable Streams Assessment, WSA, US Environmental Protection Agency 2006; Olsen and Peck 2008). The sampling design was spatially-balanced and employed an unequal probability survey with the unequal selection based on stream order.

Great river sites included in this study were the 447 sampled by the USEPA's Environmental Monitoring and Assessment Program on the eighth through tenth-order great rivers of the Upper Mississippi (144), Missouri (183), and Ohio (120) Rivers of the central USA (EMAP-GRE). Sites were sampled on the Upper Mississippi River from Lower St. Anthony Falls in Minneapolis–St. Paul, Minnesota to the confluence with the Ohio River at Cairo, Illinois; the Missouri River from Fort Peck Dam in Montana to the confluence with the Mississippi River at St. Louis, Missouri; and the Ohio River from the confluence of the Allegheny and Monongahela Rivers in Pittsburgh, Pennsylvania to the confluence with the Mississippi River (Angradi et al. 2009). The five reservoirs on the Missouri River in North and South Dakota were excluded from the survey design. The EMAP-GRE design was spatially balanced and employed an unequal probability for selecting sites based on unique river reach.

The designs for both tributary streams and great rivers surveys selected a single point along the center line as depicted by the National Hydrography Database (NHD-Plus, <http://horizon-systems.com/nhdplus>). All sample sites were selected using NHD as the sample frame. Each site included in the two surveys represented a known stream or river length, based on the population of streams and rivers included in the survey design, the probability of that site being selected for sampling, and the number of sites actually sampled. These stream and river lengths were summed to estimate the cumulative extent of streams and rivers sampled (Olsen and Peck 2008; Angradi et al. 2009).

2.2 Precipitation, Hydrology, and Catchment Land Use

Area-weighted mean annual precipitation (Ppt, mm year⁻¹) and the mean discharge throughout the year (Q_{Ann} , m³ s⁻¹, predicted from a unit runoff model) were downloaded for each site from the NHD-Plus (<http://horizon-systems.com/nhdplus>). Channel width was measured in the field (wadeable streams) or from GIS data and aerial photographs (great rivers). Tributary stream and great river depths were measured at the thalweg of each site. Total catchment area for each site was calculated by summing the areas of all NHD-Plus catchments intersected while navigating upstream from each site. Instantaneous discharge (Q_{Inst} , m³ s⁻¹) for the tributary streams was calculated as the product of wetted stream width (w , m), depth (z , m), and velocity (v , m s⁻¹; Peck et al. 2006); Q_{Inst} for the great rivers was estimated by interpolating between upstream and downstream USGS gage data, and accounting for tributary stream inflows (Richter et al. 1996).

Land cover data were extracted from the National Land Cover Database (NLCD, US Geological Survey 2001). The NLCD, derived from multi-temporal and terrain-corrected satellite imagery, provided a consistent 29-class land cover for the USA. Land cover data were aggregated into five classes for analyses: agriculture (row crop, pasture), developed (residential, commercial/industrial), wetlands, forests, and "open" (bare ground, shrub/scrublands, and grasslands). The database also included estimates of percent imperviousness and percent tree canopy. Targeted assessments found accuracies of land cover, imperviousness, and canopy ranged from 73–77%, 83–91%, and 78–93%, respectively (Homer et al. 2004).

2.3 TN and TP Concentration, Flux, and Yield

Stream water samples were single 4-L grabs from the deepest portion of the channel (the thalweg; generally near the middle of the channel). Great river water samples were cross-channel depth-integrated composites. Cross-channel transects ran perpendicular to flow in the thalweg. Combined stations included the thalweg and stations half the distance from the thalweg to each shore. Up to three depths (0.5 m from the surface and bottom and at mid depth) were

sampled at each station and proportionally added to the composite. Samples were homogenized and shipped to the lab on ice within 36 h of collection. Unfiltered sulfuric acid-preserved subsamples were digested for total nitrogen (TN) and total phosphorus (TP) using the persulfate digestion method (4500-N C and 4500-P B5, American Public Health Association 1998). Determinations of TN and TP concentrations were made by using the automated cadmium reduction method (4500-N F) and the ascorbic acid method (4500-P E), respectively (American Public Health Association 1998). Samples for $\text{NO}_3\text{-N}$ analyses were filtered (0.45 μm pore size) within 72 h of collection, preserved with H_2SO_4 , and then analyzed using the cadmium reduction method (American Public Health Association 1998). The molar ratio of TN to TP (N/P) is a reflection of the relative enrichment of N and P. Using the “Redfield ratio” (16:1, Redfield 1958) as a benchmark of balanced nutrient availability, we classified tributary stream and great river sites as N-limited (N/P < 12) or P-limited (N/P > 20).

We calculated daily TN and TP flux (kg day^{-1}) as the product of TN and TP concentrations ($\mu\text{g L}^{-1}$) and Q_{Inst} . To check the validity of using TN and TP fluxes based on summer, low flow chemistry and discharge, we compared mean summer Q_{Inst} , $\text{NO}_3\text{-N}$ flux, and TP flux with mean annual flux estimates for these variables in 21 Mississippi River basin catchments included in the US Geological Survey’s National Stream Quality Accounting Network (<http://water.usgs.gov/nasqan/>). Daily TN and TP yield ($\text{kg km}^{-2} \text{day}^{-1}$) were estimated by dividing flux by catchment area.

2.4 Reference Classification

When reference streams are available, the USEPA’s guidance for setting stream nutrient criteria recommends using the 75th percentile for the population of reference streams for setting reference condition thresholds (US Environmental Protection Agency 2000). This approach was used for wadeable streams in the Upper Mississippi, Missouri, and Ohio River basins, which included 175 reference sites based on stream chemistry (excluding N and P) and physical habitat data (Herlihy and Sifneos 2008). We incorporated their thresholds for the tributary streams in our study. We followed a similar approach for setting reference thresholds for the

great rivers. Our great rivers reference sites were the least-disturbed sites on each river, based on river chemistry (excluding N and P), physical habitat, riparian disturbances, and landscape variables. The least-disturbed stressor thresholds for each river were set using the y-intercept of the quantile regression of TN and TP against the anthropogenic stressor gradient (Angradi et al. 2009). Reference thresholds for tributary streams and great rivers were established independently for the Upper Mississippi, Missouri, and Ohio River basins.

2.5 Statistical Analyses

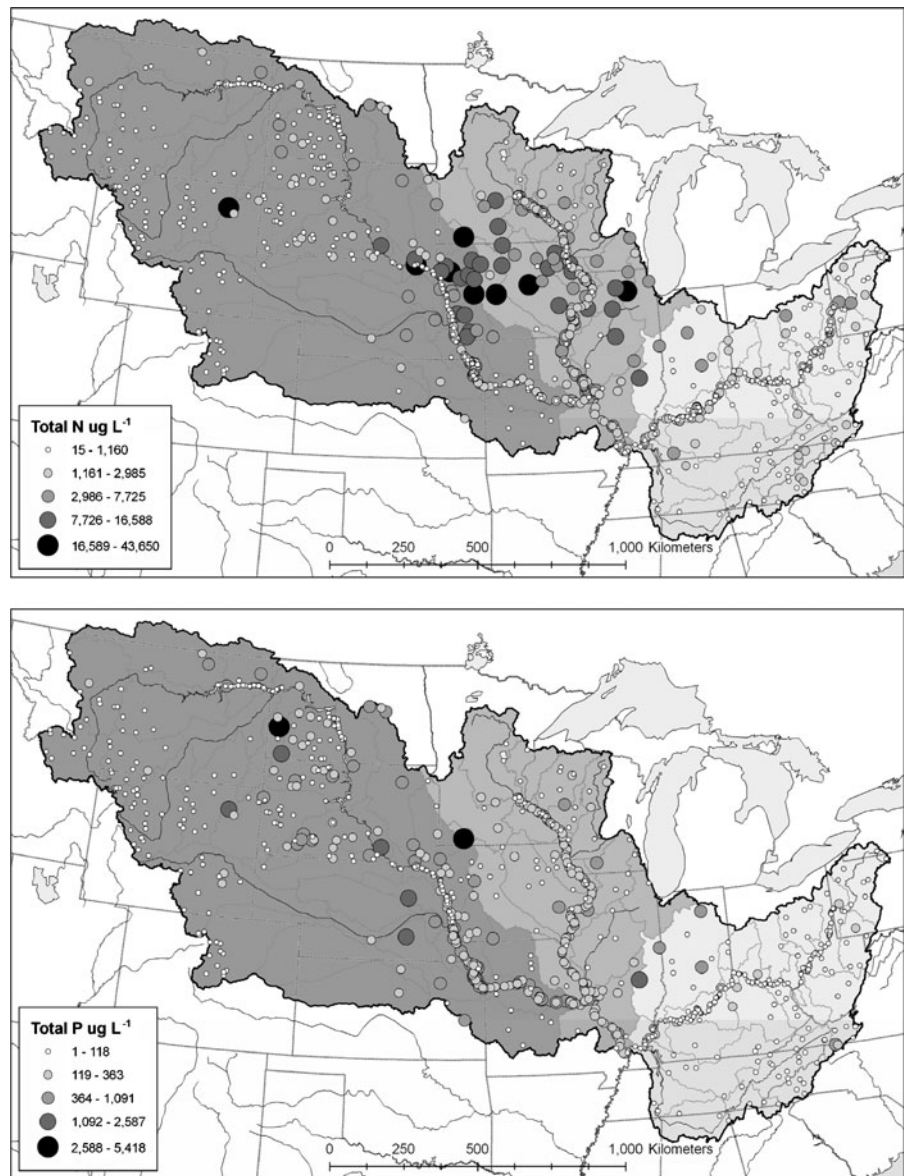
Descriptive statistics for the chemistry, hydrology, and catchment land use variables were analyzed using the SAS System for Windows 9 statistical software (Procedure MEANS, SAS Institute, Inc. Cary, NC, USA). Means were compared by basin (Upper Mississippi, Missouri, Ohio) and size class (tributary stream, great river) using the analyses of variance (Procedure ANOVA, SAS) and Sheffe’s a posteriori means tests. The data were often skewed, and the residuals from the ANOVA on untransformed data did not fit a normal distribution or have equal variances. To overcome these limitations, data were log-transformed prior to ANOVA. Comparisons of summer, low flow discharge and chemistry with annual values were done using linear regression (Procedure REG, SAS) of untransformed data.

3 Results

3.1 Site Characteristics

The 436 sampled tributary streams (Fig. 1) represent 477,064 km of streams or about 60% of the total tributary stream length within the basin. The remaining 40% of stream length, primarily intermittent and ephemeral streams not included in sample frame, was not assessed (Olsen and Peck 2008). The mean catchment size was 146 km^2 for Upper Mississippi basin tributary streams, 1,054 km^2 for Missouri basin tributary streams, and 73 km^2 for Ohio basin tributary streams (Table 1). Agricultural land use was 19% of Upper Mississippi basin tributary catchment area, 4% of Missouri basin tributary catchment area, and 15%

Fig. 1 Locations of the Upper Mississippi River basin sampling sites and their total N (*top panel*) and total P (*bottom panel*) concentrations ($\mu\text{g L}^{-1}$). The Missouri River sub-basin is shaded in *dark gray*, the Upper Mississippi River sub-basin is *medium gray*, and the Ohio River sub-basin is *light gray*



of Ohio basin tributary catchment area (Table 1). Of these, streams in the Missouri River basin drained larger catchments (ANOVA, $F=12.7$, $p<0.0001$; Table 1), and streams in the Upper Mississippi River basin drained catchments with a greater proportion of area in agriculture than did streams in the other basins (ANOVA, $F=35.9$, $p<0.0001$; Table 1). Tributary streams in the Missouri River basin received less precipitation (619 mm year^{-1}) than either the Upper Mississippi (830 mm year^{-1}) or Ohio ($1,164 \text{ mm year}^{-1}$) River basins (ANOVA, $F=215$, $p<0.0001$; Table 1).

The 447 great river sites (Fig. 1) represent 4,838 km of river channels. The Missouri River sites drained larger catchments than sites on either the Upper Mississippi or Ohio Rivers (ANOVA, $F=98.5$, $p<0.0001$; Table 1), and Upper Mississippi River sites drained catchments with a significantly greater proportion in agriculture than did the Missouri or Ohio Rivers (ANOVA, $F=629$, $p<0.0001$; Table 1). The main channel Missouri River receives less precipitation (449 mm year^{-1}) than either the Upper Mississippi (731 mm year^{-1}) or Ohio ($1,082 \text{ mm year}^{-1}$) Rivers (ANOVA, $F=3,745$, $p<0.0001$; Table 1).

Table 1 Mean values for catchment and physico-chemical attributes for tributary streams and river main channels of the Upper Mississippi, Missouri, and Ohio Rivers sub-basins

Sub-basin	A	Ag	Ppt	Q_{Inst}	Q_{Ann}	TN	TP	N:P	TN flux	TP flux	TN yield	TP yield
Upper Mississippi River basin												
Tributary streams	146 ^b	19 ^a	830 ^b	0.32 ^a	0.72 ^a	5,431 ^a	165 ^a	210 ^a	212 ^a	5.29 ^a	1.45 ^a	0.04 ^a
Great rivers	492,191	50 ^x	731 ^y	1,287 ^x	1,939 ^y	2,112 ^x	181 ^x	40.4 ^{xy}	244,899 ^x	23,284 ^x	0.50 ^x	0.05 ^x
Missouri River basin												
Tributary streams	1,054 ^a	4 ^b	619 ^c	0.35 ^a	0.82 ^a	1,751 ^b	177 ^a	58.6 ^a	94.7 ^b	9.13 ^a	0.09 ^c	0.01 ^a
Great rivers	835,249 ^x	26 ^y	449 ^z	840 ^y	1,425 ^z	978 ^y	171 ^x	20.3 ^y	141,094 ^y	16,938 ^x	0.17 ^x	0.02 ^x
Ohio River basin												
Tributary streams	73 ^b	15 ^a	1,164 ^a	0.35 ^a	0.99 ^a	1,074 ^b	66.7	156 ^b	30.8 ^c	1.62 ^a	0.42 ^b	0.02 ^a
Great rivers	208,608 ^z	27 ^y	1,082 ^x	1,314 ^x	3,124 ^x	1,152 ^y	52.6	59.9 ^x	93,886 ^y	8,147 ^y	0.45 ^x	0.04 ^x

Attributes include: catchment area (A , km²), the proportion of catchment area in agricultural (Ag, %), area-weighted mean annual precipitation (Ppt, mm year⁻¹), instantaneous (Q_{Inst}), and mean annual (Q_{Ann}) stream discharge (m³ s⁻¹), total N (TN) and total P (TP) concentrations (μg L⁻¹), molar N/P, TN, and TP flux (kg day⁻¹) and yield (kg km⁻² day⁻¹). The letters following each value indicate their grouping within tributary stream or river classes based on ANOVA and the *a posteriori* Scheffé comparison of means

Mean Q_{Inst} was similar (0.32–0.35 m³ s⁻¹) for the tributary stream sites in the Upper Mississippi, Missouri, and Ohio River basins (ANOVA, $F=0.33$, $p<0.7191$; Table 1). Mean Q_{Ann} for the tributary stream sites was also similar across basins (0.72–0.99 m³ s⁻¹; ANOVA, $F=0.33$, $p<0.7163$; Table 1). Mean Q_{Inst} in the main channel of the great river sites was 1,287 m³ s⁻¹ for the Upper Mississippi River, 840 m³ s⁻¹ for the Missouri River, and 1,314 m³ s⁻¹ in the Ohio River (ANOVA, $F=59.0$, $p<0.0001$; Table 1). Similarly, Q_{Ann} was highest in the Ohio River main channel (3,124 m³ s⁻¹) compared to the Upper Mississippi River (1,939 m³ s⁻¹) or Missouri River (1,425 m³ s⁻¹, ANOVA, $F=81.4$, $p<0.0001$; Table 1).

3.2 TN and TP Concentration, Flux, and Yield

Mean TN and TP concentrations at the tributary stream sites were 5,431 μg TN L⁻¹ and 165 μg TP L⁻¹ in the Upper Mississippi basin, 1,751 μg TN L⁻¹ and 177 μg TP L⁻¹ in the Missouri basin, and 1,074 μg TN L⁻¹ and 67 μg TP L⁻¹ in the Ohio River basin (Table 1, Fig. 1). TN concentrations were significantly greater in Upper Mississippi River tributary streams than in Missouri River or Ohio River tributary streams (ANOVA, $F=29.6$, $p<0.0001$; Table 1), but there were no significant differences among the basins for tributary TP (ANOVA, $F=2.93$, $p<0.0541$; Table 1). Both TN and TP concentrations were significantly correlated with overall catchment area and the proportion of the catchment in agriculture (Table 2, Fig. 2). TP concentrations in tributary streams were also inversely correlated with Ppt (Table 2).

Mean TN and TP concentrations at the great river sites were 2,112 μg TN L⁻¹ and 181 μg TP L⁻¹ for the Upper Mississippi River, 978 μg TN L⁻¹ and 171 μg TP L⁻¹ for the Missouri River, and 1,152 μg TN L⁻¹ and 53 μg TP L⁻¹ for the Ohio River (Table 1, Fig. 1). TN concentrations were significantly greater in the Upper Mississippi River than in the Missouri or Ohio Rivers (ANOVA, $F=105$, $p<0.0001$; Table 1), and TP concentrations were significantly lower in the Ohio River than in Missouri or Upper Mississippi Rivers (ANOVA, $F=67.8$, $p<0.0001$; Table 1). Both TN and TP concentrations were significantly correlated with the proportion of the catchment in agriculture (Fig. 2) and with Q_{Inst} ; TN was also positively correlated with Ppt; and TP was positively

Table 2 Spearman correlations (r) for tributary streams and rivers (top) and within river basins (bottom) between catchment area (A , km²), the proportion of catchment area in agricultural (Ag, %), area-weighted mean annual precipitation (Ppt, mm year⁻¹), instantaneous stream discharge (Q , m³ s⁻¹), total N (TN) and total P (TP) concentrations (µg L⁻¹), and molar N/P (NP)

	Great rivers														
	Tributary streams					Upper Mississippi River basin									
	Ag	Ppt	Q _{Inst}	TN	TP	NP	Ag	Ppt	Q _{Inst}	TN	TP	NP	Ag	Ppt	Q _{Inst}
A	-0.354	-0.537	0.405	0.218	0.296	-0.149	ns	-0.457	0.572	ns	0.486	-0.512	0.437	-0.385	0.958
Ag		0.440	-0.174	0.419	0.241	0.115	0.256	0.316	0.648	0.523	ns	ns	0.627	ns	0.451
Ppt			ns	ns	-0.267	0.282	0.184	0.250	0.354	-0.159	0.465	ns	0.627	ns	-0.383
Q _{Inst}				0.121	ns	0.184		0.458	0.458	0.465	ns	ns	0.187	ns	
TN					0.666	0.173		0.584	0.584	0.584	0.187	ns	0.187	ns	
TP						-0.573					0.584	0.187			-0.615

	Missouri River basin										Ohio River basin																	
	Upper Mississippi River basin					Missouri River basin					Upper Mississippi River basin					Ohio River basin												
	TN	TP	NP	Ag	Ppt	Q _{Inst}	TN	TP	NP	Ag	Ppt	Q _{Inst}	TN	TP	NP	Ag	Ppt	Q _{Inst}	TN	TP	NP	Ag	Ppt	Q _{Inst}	TN	TP	NP	
A	ns	0.545	-0.142	0.674	-0.253	0.876	0.366	0.456	ns	0.567	-0.218	0.901	0.435	0.513	0.138	0.567	-0.218	0.901	0.435	0.513	0.138	0.567	-0.218	0.901	0.435	0.513	0.138	
Ag	ns	0.221	ns	0.674	ns	0.717	0.351	0.451	ns	0.567	-0.153	0.531	0.440	0.478	ns	0.567	-0.153	0.531	0.440	0.478	ns	0.567	-0.153	0.531	0.440	0.478	ns	
Ppt	ns	-0.386	0.168	0.674	ns	-0.122	ns	ns	ns	0.567	ns	-0.151	-0.275	-0.204	ns	0.567	ns	-0.151	-0.275	-0.204	ns	0.567	ns	-0.151	-0.275	-0.204	ns	
Q _{Inst}	ns	0.511	ns	0.674	0.234	0.342	0.234	0.342	0.153	0.567	0.234	0.342	0.476	0.562	ns	0.567	0.234	0.342	0.476	0.562	ns	0.567	0.234	0.342	0.476	0.562	ns	
TN		-0.172	0.756	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674	0.819	ns	0.674
TP			-0.632	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674	0.819	-0.502	0.674

Only the significant ($p < 0.05$) correlations are presented

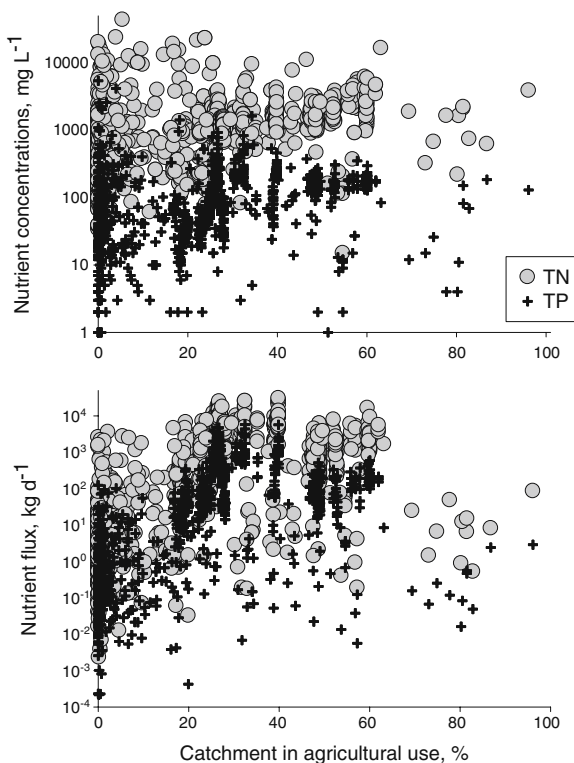


Fig. 2 Total N and P concentrations ($\mu\text{g L}^{-1}$, top panel) and flux (kg day^{-1} , bottom panel) at 914 sites in the Upper Mississippi River basin as a function of percent of their catchments in agricultural land uses

correlated with overall catchment area, and negatively correlated with Ppt (Table 2).

Mean molar N/P was highest in Upper Mississippi basin tributary streams (210:1) compared to Ohio (156:1) or Missouri (59:1) basin tributaries (ANOVA, $F=20.0$, $p<0.0001$; Table 1); for the great rivers N:P was highest in the Ohio River (60:1), followed by the Upper Mississippi (40:1) and Missouri (20:1) Rivers (ANOVA, $F=8.46$, $p<0.0002$; Table 1). In the Upper Mississippi River basin only, tributary stream N/P was significantly higher than great river N/P (ANOVA, $F=7.03$, $p<0.0074$; Table 1). N/P in the Upper Mississippi River basin was strongly correlated with both TN ($r=0.756$) and TP ($r=-0.632$), but strongly correlated only with TP in the Missouri ($r=-0.502$) and Ohio ($r=-0.466$) River basins (Table 2). Using conservative bounds ($\pm 25\%$ of the Redfield ratio), we estimated that potential N-limitation ($\text{N/P}<12$) ranged 10–21% of cumulative tributary stream length, and 0–46% of the cumulative great river length; potential P-limitation ($\text{N/P}>20$) ranged 60–83% of

cumulative tributary stream length, and 21–98% of cumulative great river length (Table 3, Fig. 3).

Mean tributary stream TN flux was highest for the Upper Mississippi basin tributaries (212 kg day^{-1}) compared to the Missouri (95 kg day^{-1}) or Ohio (31 kg day^{-1}) basin tributaries. Mean tributary TP flux was greatest in the Missouri basin (9.13 kg day^{-1}) followed the Upper Mississippi (5.29 kg day^{-1}) and Ohio (1.62 kg day^{-1}) basins. Mean TN flux was greater in the tributaries to the Upper Mississippi River than in the Missouri or Ohio River tributaries (ANOVA, $F=4.82$, $p<0.0085$), but there were no differences in TP flux among the basins (ANOVA, $F=0.74$, $p<0.4790$; Table 1). Mean river TN flux was $2.44 \times 10^5 \text{ kg day}^{-1}$ in the Upper Mississippi River, $1.41 \times 10^5 \text{ kg day}^{-1}$ in the Missouri River, and $9.39 \times 10^3 \text{ kg day}^{-1}$ in the Ohio River. Mean river TP flux in the Upper Mississippi River was $2.33 \times 10^4 \text{ kg day}^{-1}$, compared to the Missouri ($1.69 \times 10^4 \text{ kg day}^{-1}$), or Ohio ($8,147 \text{ kg day}^{-1}$) Rivers (Table 1). Mean TN flux was greater in the Upper Mississippi River than in the Missouri or Ohio Rivers (ANOVA, $F=29.7$, $p<0.0001$), and TP flux was less in the Ohio River than in the Missouri and Upper Mississippi Rivers (ANOVA, $F=13.4$, $p<0.0001$; Table 1).

Estimated TN yields from tributary stream catchments were $0.09 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Missouri basin, $0.42 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Ohio basin, and $1.45 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Upper Mississippi basin (ANOVA, $F=5.63$, $p<0.0038$; Table 1, Fig. 4). Estimated TN yields for the great river catchments were $0.17 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Missouri River, $0.45 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Ohio River, and $0.50 \text{ kg km}^{-2} \text{ day}^{-1}$ in the Upper Mississippi River (ANOVA, $F=0.10$, $p<0.9070$; Table 1, Fig. 4). Estimated TP yields from tributary stream and great river catchments were similar (0.01 – $0.04 \text{ kg km}^{-2} \text{ day}^{-1}$), and not different between basins (ANOVA, streams— $F=0.65$, $p<0.5251$; rivers— $F=0.78$, $p<0.4572$; Table 1, Fig. 4).

Comparisons of mean summer, low flow Q_{Inst} , $\text{NO}_3\text{-N}$, and TP with mean annual values for these variables at 21 Mississippi River basin catchments from 1996–2009 revealed significant positive relationships (Fig. 5). We adjusted our estimates of summer, low flow daily TN and TP flux by the inverse of the slopes of the regressions (TN 1.86; TP 1.69) before multiplying the values by 365 to estimate annual TN and TP flux (kg year^{-1}). These annual flux values were divided by the Upper Mississippi,

Table 3 Total assessed stream and river length (km) and comparisons of TN and TP reference threshold concentrations ($\mu\text{g L}^{-1}$), and the percentage of assessed tributary stream (Trib) and great river (Riv) length exceeding that threshold

	TN Threshold			%>TN threshold				TP Threshold			%>TP threshold			
	Ref	Smith	Dodds	Ref	Smith	Dodds	N_{Limited}	Ref	Smith	Dodds	Ref	Smith	Dodds	P_{Limited}
Upper Mississippi River														
Tributary streams, 102,273 km	2,500	263	222	63	94	92	11	181	40	23	48	73	84	83
Great river, 1,766 km	1,309	–	–	55	–	–	14	138	–	–	25	–	–	60
Missouri River														
Tributary streams, 99,695 km	693	102	650	50	74	56	21	68	45	66	32	60	43	60
Great river, 2,289 km	289	–	–	26	–	–	46	55	–	–	41	–	–	21
Ohio River														
Tributary streams, 202,125 km	1,336	193	314	58	80	51	10	55	28	72	33	37	37	82
Great river, 1,963 km	931	–	–	16	–	–	0	34	–	–	12	–	–	98

Reference thresholds were derived from the 75th percentiles of reference streams (Ref, Herlihy and Sifneos 2008) and reference river sites (Ref, this study), and from catchment process models (Smith et al. 2003) and land use regression models (Dodds and Oakes 2004). Our stream and river data span multiple nutrient ecoregions reported by these authors, and we used threshold values for corresponding nutrient ecoregions for each site. The cumulative percentage of stream length classified as N- or P-limited is presented

Missouri, and Ohio River catchment areas to estimate mean annual TN and TP yield ($\text{kg km}^{-2} \text{ year}^{-1}$, Table 4).

3.3 Reference Classification

There were marked differences in TN and TP reference threshold concentrations among the basins. Threshold concentrations for both TN and TP were highest for the Upper Mississippi River and its tributaries (streams—TN=2,500 $\mu\text{g L}^{-1}$, TP=181 $\mu\text{g L}^{-1}$; rivers—TN=1,309 $\mu\text{g L}^{-1}$, TP=138 $\mu\text{g L}^{-1}$), followed by the Ohio River and its tributaries (streams—TN=1,336 $\mu\text{g L}^{-1}$, TP=55 $\mu\text{g L}^{-1}$; rivers—TN=931 $\mu\text{g L}^{-1}$, TP=34 $\mu\text{g L}^{-1}$) and the Missouri River and its tributaries (streams—TN=693 $\mu\text{g L}^{-1}$, TP=68 $\mu\text{g L}^{-1}$; rivers—TN=289 $\mu\text{g L}^{-1}$, TP=55 $\mu\text{g L}^{-1}$; Table 3). The percentage of cumulative tributary stream and great river lengths exceeding TN and TP reference thresholds varied by basin: Upper Mississippi basin (streams—TN=63%, TP=48%; rivers—TN=55%, TP=25%); Missouri basin (streams—TN=50%, TP=32%; rivers—TN=26%, TP=41%); and Ohio basin (streams—TN=58%, TP=55%; rivers—TN=16%, TP=12%; Table 3).

4 Discussion

Several approaches have been proposed for setting reference condition thresholds for streams. When reference sites are identified for the population of streams and rivers being assessed, Herlihy and Sifneos (2008) recommend using the 75th percentile values of the reference distribution for TN and TP to set reference thresholds. This is the approach we used for our tributary stream and great river sites. Smith et al. (2003) used empirical models of minimally-disturbed catchments to derive natural background concentrations of TN and TP. These models, based on catchment size, runoff, atmospheric deposition, and in-stream nutrient loss rates, demonstrated the change in expected background TN and TP throughout undisturbed river networks for the conterminous USA. Dodds and Oakes (2004) used regression models of TN and TP as a function of catchment land use to set reference thresholds, which set as the intercept of the slope on nutrient concentrations for a hypothetical catchment with no agriculture or urban land uses. The general lack of consensus among these approaches highlights the differences between what modeled projections of what reference conditions

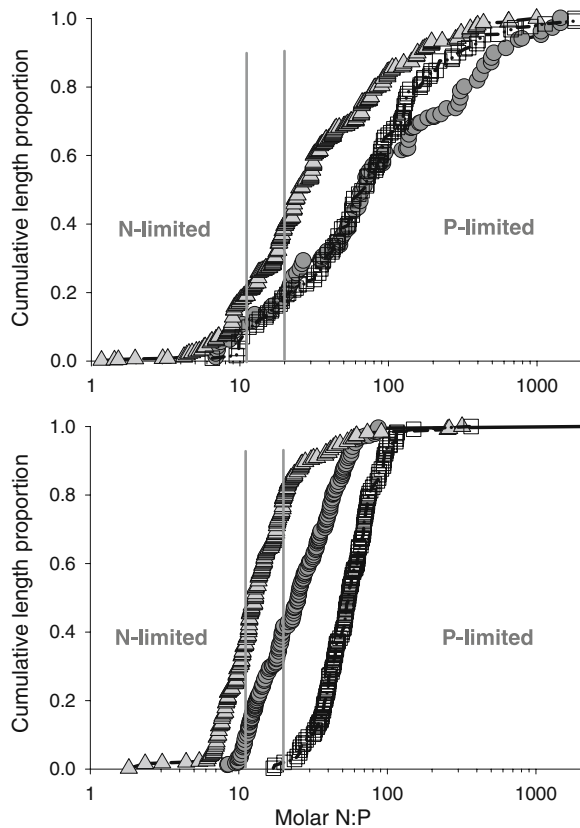


Fig. 3 Cumulative lengths of tributary streams (*top panel*) and great rivers (*bottom panel*) that are N-limited (molar N/P < 12) or P-limited (molar N/P > 20). Upper Mississippi River basin sites are indicated by *dark gray circles*, Missouri River basin sites by *light gray triangles*, and Ohio River basin sites by *open squares*

should be and what are the observed N and P concentrations in the least disturbed streams in this region (Table 3). We believe that reference thresholds based on least-disturbed sites are more defensible and likely represent more meaningful criteria for streams and rivers within a given region than are criteria based on models of undisturbed catchments.

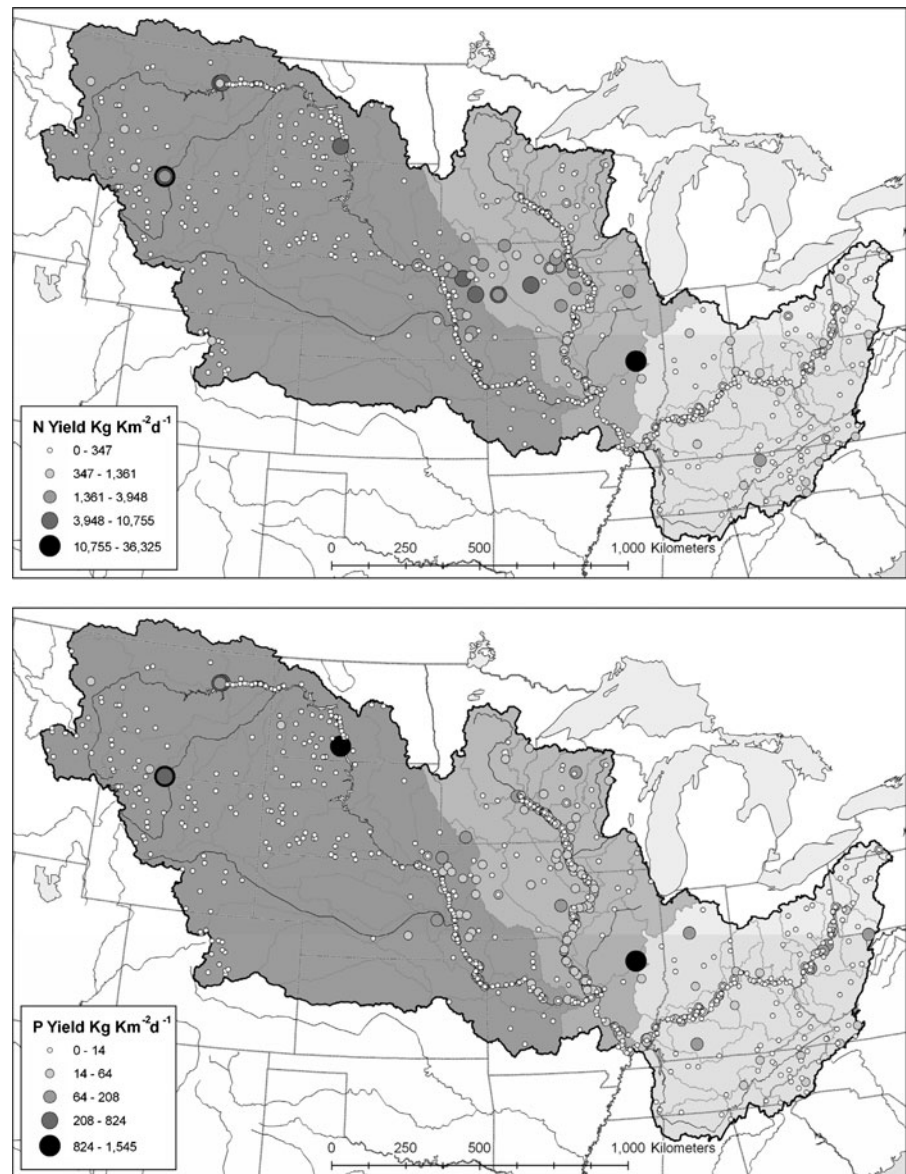
Nutrient yield estimates from a single summer site visit are constrained by the high temporal variability of nutrient concentrations and discharge. However, surveys are a valid approach for regional assessments of water quality effects on aquatic ecosystems, effects which are most apparent during summer low flow periods (Grayson et al. 1997; Dodds et al. 2009). Our estimates of TN and TP concentrations for the great rivers of the Mississippi basin are lower than the 1980–1996 averages reported for comparable river reaches (Goolsby et

al. 1999). Unlike our summer, low flow estimates, those presented by Goolsby et al. (1999) are annual averages that include spring runoff, typically a period of peak nutrient flux (Royer et al. 2006; Banner et al. 2009). $\text{NO}_3\text{-N}$ concentrations in the Mississippi River have increased over time, with reported ranges for molar N concentrations from 1899 to 1907 being 448–1,778 $\mu\text{gN L}^{-1}$, ranges from 1955 to 1965 were 1,008–2,058 $\mu\text{gN L}^{-1}$, ranges from 1980 to 1996 were 2,800–8,316 $\mu\text{gN L}^{-1}$, and ranges from 2004 to 2006 were 978–2,112 $\mu\text{gN L}^{-1}$ (Turner and Rabalais 1991; Justic et al. 1995; Goolsby et al. 1999; Broussard and Turner 2009; this study).

The absolute concentrations of TN and TP in streams is an important indicator of catchment land uses and anthropogenic nutrient loading, particularly from agriculture, but they reveal little about the potential limitation of biological processes by either of these nutrients. Stream and river N/P indicate the relative stoichiometric nutrient availability and may provide insight into relative nutrient limitation. Several factors govern N/P, including catchment area, mean annual precipitation, hydrology, and natural and anthropogenic nutrient loading (Green and Finlay 2010). Of these, hydrology and TP concentrations have been suggested as controlling factors (Green and Finlay 2010). Our data suggests that hydrology is only a weak driver of N/P, with TN (Upper Mississippi River basin only) and TP relatively more important (Table 2).

Freshwater ecosystems are often P-limited, but there is evidence that N-limitation may be more common than previously recognized (Howarth et al. 1996; Hill et al. 2010). Ecological stoichiometric theory emphasizes the importance of the balance of biologically important elements for regulating an organism's response to, and regulation of, their environment (Sterner and Elser 2002). Deviations from balanced nutrient availability may have adverse effects on biota that propagate from headwaters to downstream receiving waters (Ptacnik et al. 2005). Previous estimates of molar N/P ratios for the Mississippi River range from 9:1 to 38:1 (Justic et al. 1995; Howarth et al. 1996), while a catchment-based model estimates a ratio of 14:1 (Seitzinger et al. 2005). As was the case with $\text{NO}_3\text{-N}$ concentrations, historical changes in N/P reflect the increased N-loading to the Mississippi River basin, with N/P rising

Fig. 4 Bubble plots of total N yield (top panel, $\text{kg km}^{-2} \text{ year}^{-1}$) and total P yield (bottom panel, $\text{kg km}^{-2} \text{ year}^{-1}$) for the sub-basins of the Upper Mississippi River. The Missouri River sub-basin is shaded in *dark gray*, the Upper Mississippi River sub-basin is *medium gray*, and the Ohio River sub-basin is *light gray*



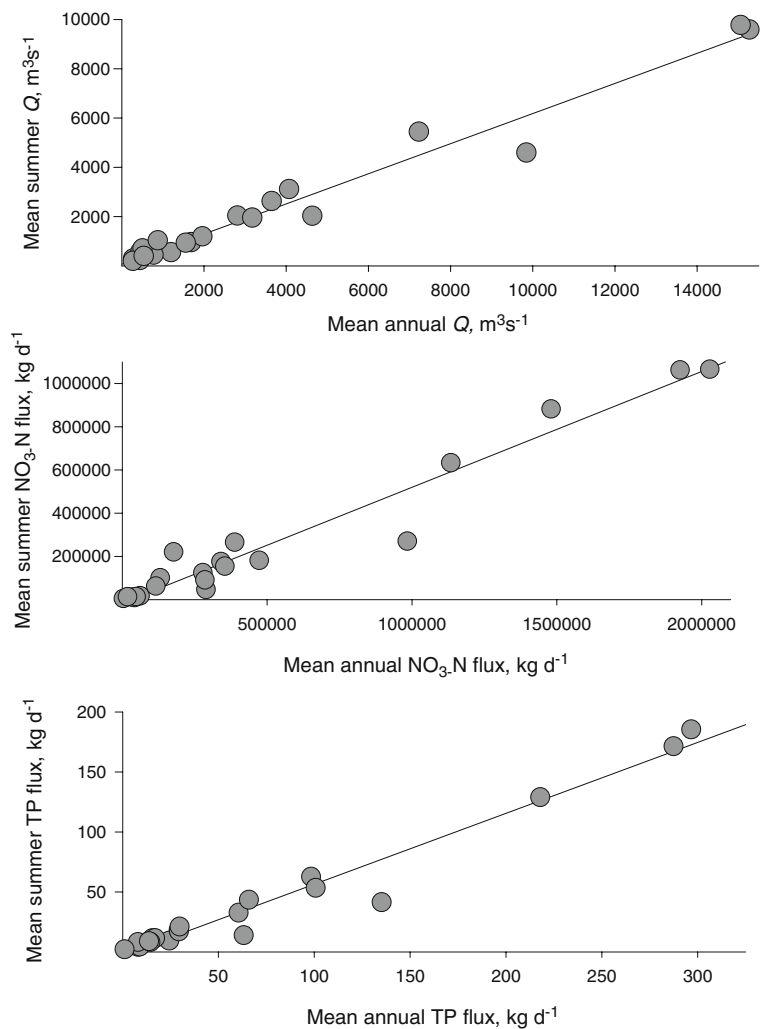
from 9:1 in 1960–1962, to 15:1 to 38:1 in 1980–1996, to 20:1 to 60:1 in 2004–2006 (Justic et al. 1995; Howarth et al. 1996; this study). This rise in N/P is most pronounced in the agriculturally intensive tributary streams of the Upper Mississippi River.

Estimated yield reveals areas where rates of TN or TP flux are greater than can be explained by catchment area. The Upper Mississippi and Ohio River basin stands out in this regard with two to three times the mean daily (or extrapolated annual) TN and TP yields compared to the Missouri River basin (Table 1, Fig. 4). Within the Upper Mississippi River basin, the streams in Iowa and northern Illinois are high TN contributors. These data

also highlight the higher TN yields from tributary streams across the Missouri, Upper Mississippi, and Ohio River basins, while no spatial pattern is evident for TP yields (Table 1, Fig. 4).

We extrapolated our estimates of daily TN and TP yields to annual estimates by adjusting our summer, low flow data based on regressions against annual estimates, then multiplying adjusted daily values by 365. Our estimates of annual TN and TP yields from our three study basins are lower than those reported by other studies of nutrient yields for the Missouri, Upper Mississippi, and Ohio River basins, suggesting that our approach for estimating annual TN and TP

Fig. 5 Regression plots of mean summer versus mean annual Q_{Inst} ($\text{m}^3 \text{s}^{-1}$, *top panel*), **b** $\text{NO}_3\text{-N}$ flux (kg day^{-1} , *middle panel*), and TP flux (kg day^{-1} , *bottom panel*) from 1996–2009 in 21 Mississippi River basin catchments. The results of the linear regressions are: mean summer $Q_{\text{Inst}} = 0.611 \times$ mean annual $Q_{\text{Inst}} + 2,552$, $r^2 = 0.97$; mean summer $\text{NO}_3\text{-N flux} = 0.536 \times$ mean annual $\text{NO}_3\text{-N flux} - 6,555$, 841 , $r^2 = 0.95$; mean summer TP flux $= 0.591 \times$ mean annual TP flux $- 1,055,769$, $r^2 = 0.96$



yields does not adequately capture high yields associated with spring runoff (Table 4).

While nutrients transported to the Gulf of Mexico come from many sources, only 22% of the N load, and 34% of the P load comes from point source discharges (Howarth et al. 1996; US Environmental Protection Agency 2007b). About 10% of the exported N is accounted for by atmospheric deposition (Howarth et al. 1996; Goolsby et al. 1999). Our data suggest that the Missouri, Upper Mississippi, and Ohio River basin are the largest contributor of both N and P to the Gulf of Mexico (Table 1). High N yields come from basins draining agricultural lands in Iowa, Illinois, Indiana, southern Minnesota, and Ohio (Goolsby et al. 1999; McIsaac et al. 2002; Alexander et al. 2008). The distribution of our sites with high TN reflects the extent of agriculture, particularly corn and soybean row

crops, in the Missouri, Upper Mississippi, and Ohio River basins; whereas the distribution of sites with high TP is indicative of sites dominated by pastures and rangeland (Goolsby et al. 1999; Turner and Rabalais 2003; Donner et al. 2004; Alexander et al. 2008; Broussard and Turner 2009; Fig. 1). Current hypoxic zone model projections suggest that a 45% reduction in both total nitrogen and total phosphorus export from the Mississippi River basin is needed to mitigate the Gulf hypoxia problem (US Environmental Protection Agency 2007b).

The Mississippi River is second only to the Amazon River in TN export to the oceans, and third overall in TP export (Howarth et al. 1996). What sets the Mississippi River apart from most major rivers is its greater percentage of its catchment in agriculture, especially corn and soybeans (Howarth et al. 1996; Goolsby et al.

Table 4 Comparisons of mean TN and TP yields ($\text{kg km}^{-2} \text{ year}^{-1}$) estimated from survey data collected in streams and rivers of the Missouri, Upper Mississippi, and Ohio River basins with published estimates based on modeling and mass balance approaches

Investigators	Approach	Basin	TN yield	TP yield
Smith et al. 1997	regression, USGS data	Missouri	–	9
		Upper Mississippi	–	55
		Ohio	–	44
Goolsby et al. 1999	mass balance, multiple sources	Missouri	180	19
		Upper Mississippi	280	18
		Ohio	940	75
Turner and Rabalais 2004	mass balance, USGS data	Missouri	140	19
		Upper Mississippi	1,120	76
		Ohio	765	68
Donner et al. 2004	process models, USGS data	Missouri	169	–
		Upper Mississippi	1,181	–
		Ohio	942	–
This study	survey data	Missouri	115	12
		Upper Mississippi	339	31
		Ohio	305	25

Annual yield estimates from this study were approximated by multiplying our low flow adjusted (TN flux \times 1.86; TP flux \times 1.69) estimates of daily yield estimates by 365

1999; Donner et al. 2004). Overall, more than 30% of the land use in the Mississippi's catchment is agriculture, with some of the major basins exceeding 50% agricultural land use. Fertilizer application (ca. $2,000 \text{ kg km}^{-2} \text{ year}^{-1}$) and N-fixation by leguminous plants (ca. $1,000 \text{ kg km}^{-2} \text{ year}^{-1}$) account for the majority of N inputs to the Mississippi River's catchment, of which approximately half, 1.3 to $1.7 \times 10^6 \text{ T year}^{-1}$, are delivered to the Gulf of Mexico (Howarth et al. 1996; Goolsby et al. 1999; Rabalais et al. 2002; Alexander et al. 2008). The remaining N input is stored in catchment soils and sediments, exported as food and fiber commodities, or reduced by denitrification.

The recent emphasis on sustainable energy production, and the corresponding increase in ethanol production, has resulted in a 19% increase in the amount of land devoted to corn cultivation within the Mississippi River basin (US Department of Agriculture 2008). Much of this increase in cultivation has been on lands that were previously deemed marginal for row crops because of poor soil quality or steep slopes (US Environmental Protection Agency 2007b; National Research Council 2008). These marginal lands often require heavy fertilizer applications to achieve high yields. Because they are often steeply sloped, they experience significant losses of fertilizer through runoff (US Environmental Protection Agency

2007b; National Research Council 2008). The present study does not address the impact of this increased corn cultivation on nutrient export or Gulf of Mexico hypoxia. However, it documents, using extensive empirical data, the link between the extent of agriculture in a catchment and the nutrient chemistry of the streams that drain those catchments. This study also demonstrates that monitoring data, especially those derived from probability surveys, may be used for estimating nutrient yields and export, and thus provide a baseline against which future surveys of tributary streams and great rivers in the Mississippi River basin may be compared.

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