Biogeochemistry (2011) 102:183–194
DOI 10.1007/s10533-010-9431-8

Nitrogen removal by streams and rivers of the Upper Mississippi River basin

Brian H. Hill · David W. Bolgrien

Received: 17 September 2009 / Accepted: 12 March 2010 / Published online: 4 April 2010

Abstract We used stream chemistry and hydrogeomorphology data from 549 stream and 447 river sites to estimate NO_3–N removal in the Upper Mississippi, Missouri, and Ohio Rivers. We used two N removal models to predict NO_3–N input and removal. NO_3–N input ranged from 0.01 to 338 kg km^{-1} d^{-1} in the Upper Mississippi River to <0.01–54 kg km^{-1} d^{-1} in the Missouri River. Cumulative river network NO_3–N input was 98700–101676 Mg $year^{-1}$ in the Ohio River, 85961–89288 Mg $year^{-1}$ in the Upper Mississippi River, and 59463–61541 Mg $year^{-1}$ in the Missouri River. NO_3–N output was highest in the Upper Mississippi River (0.01–329 kg km^{-1} d^{-1}), followed by the Ohio and Missouri Rivers (<0.01–236 kg km^{-1} d^{-1}) sub-basins. Cumulative river network NO_3–N output was 97499 Mg $year^{-1}$ for the Ohio River, 84361 Mg $year^{-1}$ for the Upper Mississippi River, and 59200 Mg $year^{-1}$ for the Missouri River. Proportional NO_3–N removal (PNR) based on the two models ranged from <0.01 to 0.28. NO_3–N removal was inversely correlated with stream order, and ranged from <0.01 to 8.57 kg km^{-1} d^{-1} in the Upper Mississippi River to <0.001–1.43 kg km^{-1} d^{-1} in the Missouri River. Cumulative river network NO_3–N removal predicted by the two models was: Upper Mississippi River 4152 and 4152 Mg $year^{-1}$, Ohio River 3743 and 378 Mg $year^{-1}$, and Missouri River 2277 and 197 Mg $year^{-1}$. PNR removal was negatively correlated with both stream order ($r = -0.80$–0.87) and the percent of the catchment in agriculture ($r = -0.38$–0.76).

Keywords Nitrogen removal · River networks · Mississippi River basin

B. H. Hill (✉) · D. W. Bolgrien
Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804, USA
e-mail: hill.brian@epa.gov

Introduction

Riverine ecosystems around the world are impacted by the development of their catchments for human uses. Conversions of catchments to agricultural, urban, and industrial land uses, 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). Loadings of excess nutrients and sediment to rivers and streams from non-point sources are the leading causes of water quality degradation in the United States (Turner and Rabalais 2003; Broussard and Turner 2009). One consequence of increasing the extent of agricultural lands in the Mississippi River basin is increased N loading to streams that has increased N exports to the Gulf of Mexico. A result has been the development of a persistent hypoxic zone (Donner et al. 2004; Turner et al. 2008). Globally, the Mississippi River basin is second only to the Amazon basin in total N export to the ocean (Howarth et al. 1996). What sets the Mississippi apart from other major rivers is the percentage of its basin that is in row-crop agriculture; especially corn and soybeans (Howarth et al. 1996; Goolsby et al. 1999; Donner et al. 2004). The positive relationship between the proportion of row-crop agriculture in a river's basin and the NO_3–N concentrations in its rivers and streams is widely recognized. Overall, more than 30% of the Mississippi River basin is in agriculture, with some sub-basins exceeding 50%. Roughly half of the N attributable to fertilizer application and N-fixation by leguminous plants is retained within the basin, exported as food and fiber commodities, or lost through denitrification. The balance of the N is delivered to the Gulf of Mexico (Howarth et al. 1996; Goolsby et al. 1999; Rabalais 2002; Alexander et al. 2008). It is this fraction that is the focus of actions to reduce the size of the hypoxic zone (Mississippi River/Gulf of Mexico Nutrient Task Force 2001).

The basic strategies for reducing hypoxia in the Gulf of Mexico are to reduce N sources in the basin and reduce the amount of N delivered to the Gulf (Mississippi River/Gulf of Mexico Nutrient Task Force 2001). Reductions can be achieved by increasing the retention of N in soils, sediments and biomass, reducing atmospheric and terrestrial N (largely inorganic agricultural fertilizers) sources, and increasing in-stream N removal and retention processes. The spatial scales of these approaches vary from continental (e.g. atmospheric deposition) to the local (e.g. riparian buffer strips adjacent to agricultural fields). No single approach is expected to affect enough N reduction to mediate Gulf hypoxia, but enhancing N removal by river networks will help achieve this goal.

In-stream N removal involves ammonia volatilization, denitrification, and biomass removal; N retention includes sequestration in biomass or sediment. When N loading exceeds the capacities of removal or retention processes the excess is exported downstream (Bernot and Dodds 2005; Burgin and Hamilton 2007). Removal and retention processes occur at every downstream stage, including in our case the Gulf of Mexico. Modeled and empirical estimates of the effectiveness of removal and retention mechanisms must account for N availability (supply, uptake, and transport) and stream hydrogeomorphology (Bernot and Dodds 2005; Earl et al. 2006). Hydrogeomorphology, in particular stream size, is important because it determines the potential for N (specifically, NO_3–N) to interact in biological processes either in the water column or sediments that can result in its removal or retention. A comparison of river network-scale nutrient removal models indicated that the capacity of streams to remove NO_3–N, thus ameliorating downstream export, increased when stream and river network size (e.g. stream or reach length, stream order) were considered (Wollheim et al. 2006). Similarly, Ensign and Doyle (2006) found N removal capacity increased with stream order. These studies highlight the relative importance of large rivers for NO_3–N removal at the network-scale.

Using empirical N removal data from 72 streams across diverse biomes and a model for the 5th-order Tennessee River, Mulholland et al. (2008) found that as NO_3–N concentrations increased, absolute rates of removal generally increased but the efficiency of removal (i.e. proportional amount removed to the amount available) decreased exponentially. Key to those trends was the relationship between NO_3–N availability and N uptake velocity (V_f–NO_3; cm s^{-1}). V_f–NO_3, the vertical velocity at which a molecule of NO_3–N moves through the water column toward the sediments, is a measure of biotic NO_3–N uptake as assimilation or denitrification. It is also considered the mass transfer coefficient. A meta-analysis by Tank et al. (2008) corroborated ambient N concentration (NO_3–N) as a significant predictor of V_f–NO_3. This relationship was consistent across ecoregions and stream types (Mulholland et al. 2008). That V_f is linked to NO_3–N availability and remains relatively constant across stream types and sizes, simplifies prediction of potential N removal by river networks (Ensign and Doyle 2006; Wollheim et al. 2006; Mulholland et al. 2008).

Results from Mulholland et al. (2008) also underscore the importance of hydrogeomorphology throughout a stream network for controlling net export of NO_3–N. They found that at low N loads, shallow low-order streams account for most N removal in networks. Higher-order streams essentially remain N limited. Alexander et al. (2000) used a mass-balance approach to describe this inverse relationship between N removal and stream depth. As N loads increase, small streams become N saturated and export excess N downstream to larger streams with capacity to remove it. Eventually, N availability reaches levels that render N removal inefficient for all stream orders. Similar results were reported by Wollheim et al. (2006). The importance of accounting for N removal relative to N availability across all channel sizes highlights the need for comparative studies across a range of stream sizes (Seitzinger 2008).

These relationships suggest that N removal across a large river basin, such as the Mississippi River, can be predictable from NO_3–N concentrations, hydrogeomorphology (depth, width, velocity), and network topology (stream order) data. Such analyses can be limited by the availability of data that are comparable and representative of streams across all orders, size classes, and watershed land use characteristics, Our study takes advantage of survey data collected to assess water quality and biological condition of streams and rivers of the U.S. including the Missouri, Ohio, and Upper Mississippi Rivers (Olsen and Peck 2008; Angradi et al. 2009). Our objective is to use measured stream chemistry and hydrogeomorphology in N removal models to predict NO_3–N removal in the Mississippi River basin. We use N removal models from Seitzinger et al. (2002) and Wollheim et al. (2006) to investigate the importance of NO_3–N availability and stream size for regulating NO_3–N uptake at a regional scale, and to estimate cumulative NO_3–N removal for river networks in the Mississippi River basin.

Methods

Site selection

We obtained data from two independent surveys of streams and rivers conducted by the US EPA's Environmental Monitoring and Assessment Program (EMAP). Both surveys used spatially balanced, unequal probability designs to select sampling sites. The designs ensured unbiased and representative sampling of stream order (stream sites) and river reaches (river sites). The designs selected a single point along the center line as defined by the National Hydrography Database (NHD-Plus, http://horizon-systems.com/nhdplus). All sampling was done in relation to this point.

We included 549 1st–8th order (Strahler 1957) stream sites in the Mississippi River basin that were sampled for the assessment of streams of the conterminous United States (the Wadeable Streams Assessment, or WSA, US Environmental Protection Agency 2006; Olsen and Peck 2008, Fig. 1). We also used the 447 Upper Mississippi, Missouri, and Ohio Rivers sites sampled by the US EPA's Environmental Monitoring and Assessment Program on the great rivers of the central United States (EMAP-GRE; Angradi et al. 2009, Fig. 1).

Stream chemistry

We collected a single 4-l grab water sample from the middle (usually the thalweg) of the channel for each stream site. For river sites, we combined up to nine sub-samples (three stations across the channel × three depths) into a single sample for each site (Angradi et al. 2009). All sub-samples were combined proportionally. Samples were shipped to labs on ice within 36 h of collection. Sub-samples for NO_3–N analyses were filtered (0.45 m 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).

River networks, hydrology, and land use

We derived river networks, flowlines, and stream orders (1st–9th or 10th order) for the Upper Mississippi, Missouri, and Ohio River sub-basins from the National Hydrologic Database (NHD-Plus, http://www.horizon-systems.com/nhdplus/). We summed stream lengths by stream order and by sub-basin. Channel width (w, m) for stream sites was measured in the field (Kaufmann et al. 1999). Since w for river sites could not be accurately measured in the field, we determined w from the National Hydrologic Database and confirmed it using aerial photography. Channel depth (z, m) for stream and river sites was measured at the thalweg. We calculated instantaneous discharge (Q, $m^3\ s^{-1}$) for streams as the product of w, z, and velocity (v, $m\ s^{-1}$). We estimated Q for the river sites by interpolating between upstream and downstream US Geological Survey (USGS) gage data, and accounting for tributary inflows.

We gathered land cover information from the National Land Cover Database (NLCD, US Geological Survey 2001). The NLCD data were derived

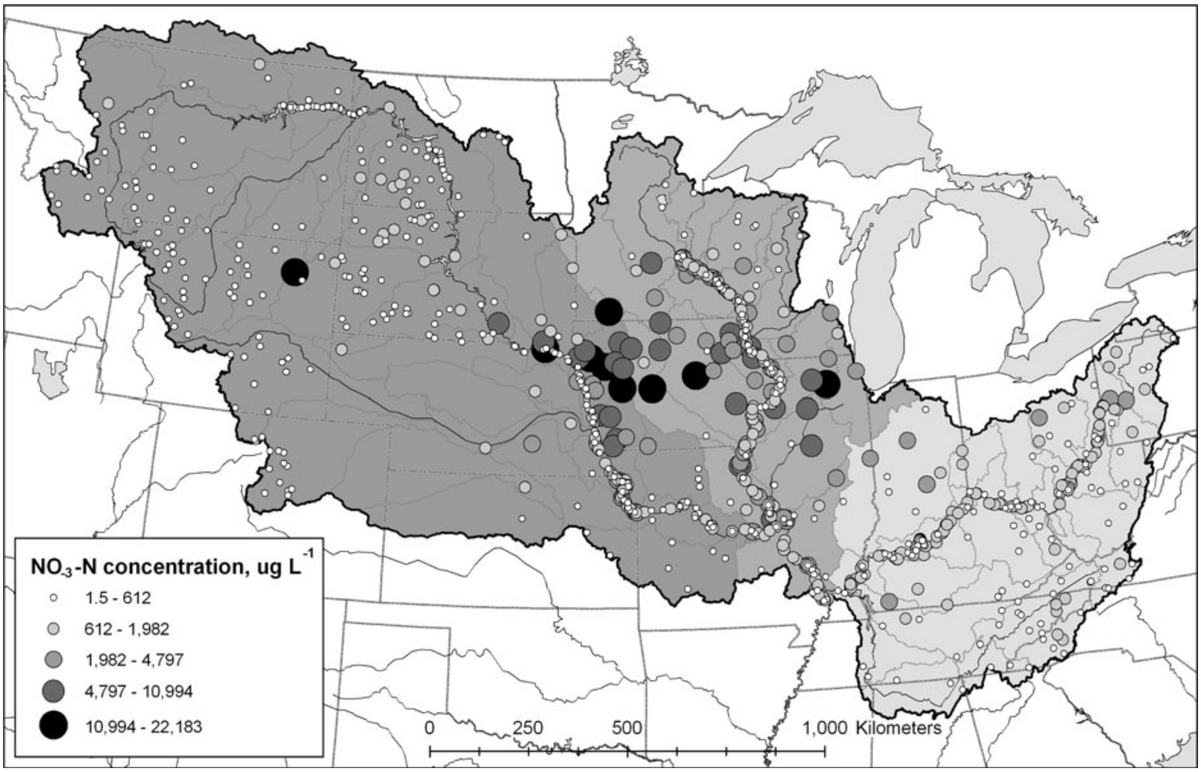

Fig. 1 NO_3–N concentrations (μg L^{-1}) at each sampling site in the Upper Mississippi River basin. The Missouri River basin is shaded *dark gray*; the Upper Mississippi River basin *medium gray*; and the Ohio River basin *light gray*

from multi-temporal and terrain-corrected satellite imagery, and classified into a consistent 29-class land cover for the United States. The NLCD has a reported accuracy of 73–77% (Homer et al. 2004).

Nitrate input, output and removal

We estimated potential NO_3–N removal by combining known relationships between NO_3–N uptake with measured NO_3–N concentrations, *z*, *w*, and *Q* (Seitzinger et al. 2002; Wollheim et al. 2006; Mulholland et al. 2008) to calculate NO_3–N input and output to a stream reach (Table 1). Here "output" refers to the ambient or measured mass of NO_3–N in a standard 1 km stream reach (kg km^{-1} d^{-1}) while "input" refers to the mass of NO_3–N in 1 km of stream predicted to exist in the absence of NO_3–N removal processes (Seitzinger et al. 2002). We applied the regression model of Mulholland et al. (2008) to our NO_3–N data to estimate V_f–NO_3 at all 996 sites in the Mississippi River basin (Table 1). We used two models to estimated proportional NO_3–N removal. The Wollheim et al. (2006) model estimates proportional N removal (PNR_W) as a function of V_f–NO_3 and hydraulic load (H_L, estimated as Q/wl; Table 1). Hydraulic load is stream velocity (m s^{-1}) relative to the benthic surface area (Mulholland et al. 2008).The Seitzinger et al. (2002) model estimates proportional N removal (PNR_S) as a product of an empirical constant and z/τ, where τ is the hydraulic residence time ($1/v$, Table 1). We calculated NO_3–N input per km of stream (kg km^{-1} d^{-1}) for the Seitzinger (NIn_S) and Wollheim (NIn_W) models as the product of N output and the reciprocal of PNR_x, where x designates either the Seitzinger (S) or Wollheim (W) models (Table 1). Finally, we estimated cumulative river network NO_3–N inputs, outputs, and removal ($CNIn_X$, $CNOut_X$, and CNR_X, Mg $year^{-1}$) as the product of NIn_x or $NOut_x$ and cumulative stream length (*L*, km; Table 1).

Table 1 Definitions of the physical dimensions of the study sites and the NO_3–N uptake metrics

Label	Name	Units	Derivation	Reference
L	Cumulative stream length	km		
l	Reach length	km		
z	Stream depth	m		
w	Stream width	m		
v	Stream velocity	$m\ s^{-1}$		
Q	Stream discharge	$m^3\ s^{-1}$		
H_L	Hydraulic load	m	$Q/w * l$	Wollheim et al. (2006)
τ	Residence time	h	$1/v$	Wollheim et al. 2006
NO_3–N	Nitrate–N concentration	$\mu g\ L^{-1}$		
V_f–NO_3	NO_3–N uptake velocity	$cm\ s^{-1}$	$(-0.462 * \log_{10} [NO_3–N]) - 2.206$	Mulholland et al. (2008)
N_{Out}	NO_3–N output	$g\ km^{-1}\ d^{-1}$	$NO_3–N * Q * l$	
PNR_W	Proportional NO_3–N removal	Unitless	$1 - Exp\ (-V_f/H_L)$	Wollheim et al. (2006)
PNR_S	Proportional NO_3–N removal	Unitless	$74.16 * (z/\tau)^{-0.344}$	Seitzinger et al. (2002)
NIn_W	NO_3–N input, Wollheim model	$g\ km^{-1}\ d^{-1}$	$N_{Out} * (1/PNR_W)$	Seitzinger et al. (2002)
NIn_S	NO_3–N input, Seitzinger model	$g\ km^{-1}\ d^{-1}$	$N_{Out} * (1/PNR_S)$	Seitzinger et al. (2002)
CN_{Out}	Cumulative river network N output	$Mg\ year^{-1}$	$N_{Out} * L * 365$	
$CNIn_W$	Cumulative river network N input, Wollheim model	$Mg\ year^{-1}$	$NIn_W * L * 365$	
$CNIn_S$	Cumulative river network N input, Seitzinger model	$Mg\ year^{-1}$	$NIn_S * L * 365$	
CNR_W	Cumulative river network NO_3–N removal, Wollheim model	$Mg\ year^{-1}$	$(NIn_w–N_{Out}) * L * 365$	
CNR_S	Cumulative river network NO_3–N removal, Seitzinger model	$Mg\ year^{-1}$	$(Nin_S–N_{Out}) * L * 365$	

Statistical analyses

Descriptive statistics for the geomorphology and hydrology metrics, and NO_3–N concentrations and uptake variables by stream order were analyzed using the MEANS procedure in the SAS System for Windows 9 statistical software (SAS Institute, Inc. Cary, NC, USA). Missing data from 6th through 8th order Upper Mississippi and Ohio sub-basin streams were estimated using the slope and intercept of linear regressions of the log-transformed variable of interest against stream order using SAS's REG procedure.

Results

Streams and rivers of the Mississippi River basin spanned a broad range of NO_3–N concentrations (Table 2, Fig. 1), channel sizes, and hydrological characteristics (Table 2). The lower order (1st–4th order) streams of the Missouri and Ohio sub-basins were deeper, wider, and had greater Q than their counterparts in the Upper Mississippi River sub-basin; z, w, and Q in higher order (>5th order) streams and rivers of the Ohio River sub-basin were greater than in their counterparts on the Missouri River sub-basin, and comparable to those in the Upper Mississippi River sub-basin (Table 2).

Rivers and streams of the Upper Mississippi River sub-basin had the highest NO_3–N by stream order than in the other sub-basins (Table 2, Fig. 2a). Differences were greatest in 1st–4th order streams where NO_3–N in the Upper Mississippi River sub-basin was 2–10 times greater than in other sub-basins. Reflecting the inverse relationship of V_f–NO_3 to NO_3–N availability, V_f–NO_3 was highest in the Missouri River sub-basin and lowest in the Upper Mississippi River sub-basin. Within a sub-basin,

Table 2 Mean stream depth (z, m), width (w, m), velocity (v, m s^{-1}), discharge (Q, m^3 s^{-1}), hydraulic load (HL, m), hydraulic residence time (τ, h), NO_3–N concentrations (μg N L^{-1}), and mass transfer rates via NO_3–N removal (V_f–NO_3, cm s^{-1}) for streams and rivers of the Mississippi River basin

Order	n	z	w	v	Q	HL	t	NO_3–N	V_f–NO_3
Upper Mississippi River									
1st	11	0.32	2.70	0.02	0.02	0.01	0.017	4137	0.125
2nd	31	0.36	4.92	0.08	0.20	0.04	0.024	3091	0.072
3rd	16	0.43	6.67	0.06	0.16	0.03	0.069	2949	0.073
4th	14	0.59	14.4	0.10	0.94	0.06	0.041	4259	0.050
5th	1	0.84	24.1	0.07	1.36	0.06	0.004	3035	0.027
[a]6th	–	3.01	100	1.15	282	1.48	0.014	2374	0.062
[a]7th	–	3.73	212	1.18	748	1.67	0.009	2023	0.059
[a]8th	–	4.63	448	1.21	1987	1.87	0.005	1672	0.055
9th	143	5.00	1551	0.17	866	0.83	0.003	1270	0.054
10th	29	6.27	753	0.78	3333	4.90	0.001	1064	0.050
Missouri River									
1st	20	0.08	2.85	0.17	0.07	0.02	0.003	233	0.160
2nd	56	0.15	7.20	0.16	0.20	0.03	0.001	579	0.151
3rd	86	0.21	10.8	0.14	0.30	0.03	0.012	1469	0.136
4th	58	0.22	17.2	0.11	0.51	0.04	0.006	1112	0.104
5th	27	0.28	33.4	0.11	1.82	0.06	0.006	1201	0.086
6th	5	0.17	60.9	0.03	0.34	0.01	0.008	759	0.081
7th	14	0.22	78.8	0.04	0.75	0.01	0.007	352	0.119
8th	32	2.44	344	0.35	199	0.72	0.001	49.2	0.292
9th	181	4.10	364	0.80	951	3.39	0.001	491	0.140
Ohio River									
1st	35	0.20	2.63	0.11	0.06	0.02	0.011	357	0.122
2nd	40	0.34	5.42	0.05	0.07	0.01	0.036	624	0.127
3rd	21	0.47	10.6	0.07	0.36	0.04	0.007	619	0.097
4th	8	0.90	16.0	0.15	2.06	0.13	0.003	699	0.076
5th	2	1.03	26.9	0.15	4.51	0.17	0.002	242	0.094
[a]6th	–	4.69	110	1.19	86.3	2.00	0.009	720	0.072
[a]7th	–	6.35	229	1.22	246	2.34	0.007	762	0.062
8th	123	8.91	506	0.24	967	1.96	0.002	793	0.052
9th	25	8.10	965	0.44	3122	3.26	0.001	860	0.056

Physico-chemical attributes for stream orders designated as [a]xth were estimated using linear regression of log-transformed stream and river data against stream order; n = the number of sites in each stream order

V_f–NO_3 generally decreased with increasing stream order (Table 2, Fig. 2b).

NO_3–N input increased with increasing stream order in all three sub-basins, ranging from <0.001 kg km^{-1} d^{-1} in many of the smaller tributary streams to 338 kg km^{-1} d^{-1} in the 10th order Mississippi River (Table 3). Estimated N input was greater for the Seitzinger model (NIn_S) than for the Wollheim model (NIn_W; Table 3). Cumulative N input was highest in both models for the Ohio River sub-basin ($CNIn_W$ 98700 Mg $year^{-1}$; $CNIn_S$ 101676 Mg $year^{-1}$) compared to either the Mississippi River ($CNIn_W$ 85961 Mg $year^{-1}$; $CNIn_S$ 89288 Mg $year^{-1}$) or Missouri River ($CNIn_W$ 59463 Mg $year^{-1}$; $CNIn_S$ 61541 Mg $year^{-1}$; Table 3).

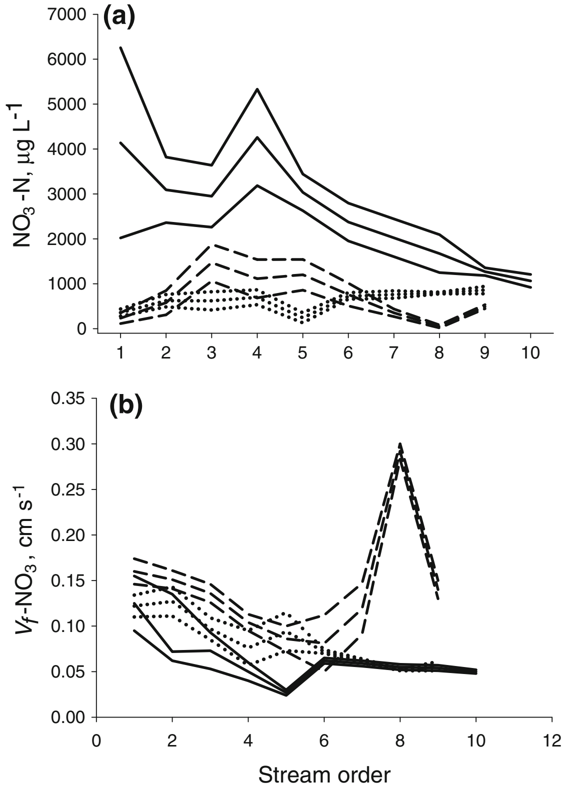

Fig. 2 Mean (±SE) NO_3–N concentrations (**a**, μg L^{-1}) and V_f–NO_3 (**b**, cm s^{-1}) by stream order for the Upper Mississippi River basin (*solid lines*), Missouri River basin (*dashed lines*), and the Ohio River basin (*dotted lines*). The center line of each triplet of lines is the mean bounded by the upper and lower SE of the mean

NO_3–N output (N_{Out}) also increased with increasing stream order and was highest in the Upper Mississippi River sub-basin (0.01–329 kg km^{-1} d^{-1}) and lowest in the Missouri River sub-basin (<0.01–52.7 kg km^{-1} d^{-1}; Table 3, Fig. 3). Despite its overall greater cumulative stream length, the Missouri River sub-basin had a lower cumulative N_{Out} (CN_{Out}, 59200 Mg $year^{-1}$) than either the Upper Mississippi

Table 3 Total stream length (L, km), and mean NO_3–N output (N_{Out}, g km^{-1} d^{-1}), proportional NO_3–N removal using the Wollheim (PNR_W) and Seitzinger (PNR_S) models, NO_3–N input (g km^{-1} d^{-1}) based on the Wollheim (NIn_W) and Seitzinger (NIn_S) models, NO_3–N removal (g km^{-1} d^{-1}) based on the Wollheim (NR_W) and Seitzinger (NR_S) models, cumulative river network NO_3–N output (CN_{Out}, Mg year^{-1}), cumulative river network NO_3–N input (Mg year^{-1}) based on the Wollheim ($CNIn_W$) and Seitzinger ($CNIn_S$) models, and cumulative river network NO_3–N removal (Mg year^{-1}) based on the Wollheim et al. (CNR_W, 2006) and Seitzinger et al. (CNR_S, 2002) models for streams and rivers of the Mississippi River basin

	L	N_{Out}	PNR_W	PNR_S	NIn_W	NIn_S	NR_W	NR_S	CN_{Out}	$CNIn_W$	$CNIn_S$	CNR_W	CNR_S
Upper Mississippi River													
1st	213,657	0.01	0.23	0.28	0.01	0.01	<0.01	<0.01	645	723	919	13.4	209
2nd	65,384	0.11	0.14	0.23	0.13	0.14	<0.01	0.01	2519	3033	3329	9.80	306
3rd	36,893	0.07	0.21	0.27	0.08	0.09	<0.01	0.01	972	1073	1163	46.9	136
4th	19,905	0.37	0.08	0.17	0.37	0.41	<0.01	0.04	2692	2710	2998	17.0	305
5th	11,055	0.36	<0.01	0.12	0.36	0.41	<0.01	0.05	1439	1446	1635	6.93	196
[a]6th	4,863	10.0	0.08	0.14	10.0	10.3	1.03	2.37	1517	1762	1722	8.02	173
[a]7th	1,827	18.6	0.05	0.11	18.6	19.4	1.04	3.01	2938	3251	3251	8.11	258
[a]8th	1,673	34.7	0.03	0.08	34.7	36.3	1.04	3.82	5689	5998	6138	8.20	386
9th	490	115	<0.01	0.06	115	120	0.06	5.60	20489	20500	21490	11.0	1001
10th	378	329	<0.01	0.03	330	338	0.03	8.57	45461	45465	46643	3.96	1182
Sub-basin total													
	355,684	–	–	–	–	–	–	–	84361	85961	89288	133	4152
Missouri River													
1st	619,339	<0.01	0.27	0.27	<0.01	<0.01	<0.01	<0.01	486	501	562	15.2	75.9
2nd	178,987	<0.01	0.18	0.23	0.01	0.01	<0.01	<0.01	363	484	451	91.3	58.7
3rd	104,200	0.05	0.24	0.26	0.05	0.05	<0.01	0.01	1787	1829	2018	41.4	231
4th	54,512	0.08	0.22	0.28	0.09	0.09	<0.01	0.01	1680	1732	1882	16.7	167
5th	27,882	0.27	0.17	0.24	0.27	0.30	<0.01	0.03	2747	2758	3019	11.8	272
6th	13,969	0.02	0.13	0.27	0.02	0.03	<0.01	0.01	108	118	147	9.69	39.0
7th	6,117	0.02	0.13	0.23	0.02	0.03	<0.01	0.01	45.0	48.6	58.0	3.64	13.0
8th	1,245	0.87	<0.01	0.01	0.87	0.91	<0.01	0.04	394	395	414	0.77	20.0
9th	2,688	52.69	<0.01	0.03	52.6	54.0	<0.01	1.43	51590	51597	52990	6.40	1400
Sub-basin total													
	1,008,939	–	–	–	–	–	–	–	59200	59463	61541	197	2277
Ohio River													
1st	250,615	<0.01	0.22	0.27	<0.01	<0.01	<0.01	<0.01	109	117	133	5.35	21.4
2nd	71,477	0.01	0.24	0.27	0.02	0.01	0.01	<0.01	153	499	211	325	36.9
3rd	41,174	0.02	0.06	0.17	0.02	0.03	<0.01	<0.01	340	375	428	4.50	57.1
4th	23,621	0.10	<0.01	0.09	0.10	0.11	<0.01	0.01	834	838	919	3.80	84.6
5th	12,596	0.12	0.01	0.09	0.12	0.13	<0.01	0.01	561	563	605	2.20	44.4
[a]6th	6,234	14.2	0.06	0.12	14.3	14.5	1.01	2.26	3388	3715	3776	8.83	257
[a]7th	2,053	27.3	0.03	0.09	27.4	28.0	1.01	2.77	9772	10233	10544	10.0	525
8th	2,419	72.3	<0.01	0.04	72.4	74.8	0.02	2.45	63874	63889	66041	15.3	2167
9th	214	236	<0.01	0.03	236	243	0.03	7.04	18468	18471	19019	2.63	550
Sub-basin total													
	410,103	–	–	–	–	–	–	–	97499	98700	101676	378	3743
Basin total													
	1,774,176	–	–	–	–	–	–	–	241060	244124	252505	708	10177

Output, input, and removal attributes for stream orders designated as [a]xth were estimated using linear regression of log-transformed stream and river data against stream order

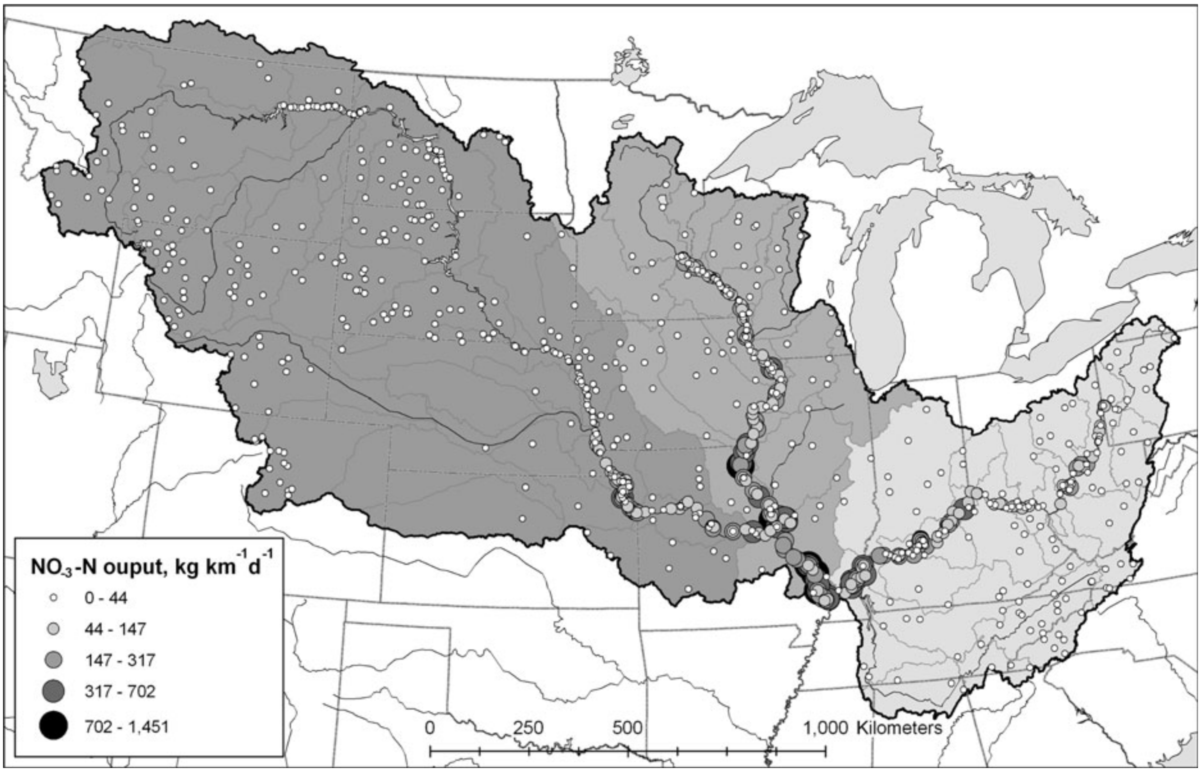

Fig. 3 NO_3–N output (kg km^{-1} d^{-1}) at each sampling site in the Upper Mississippi River basin. The Missouri River sub-basin is shaded in *dark gray*; the Upper Mississippi River sub-basin is *medium gray*; and the Ohio River sub-basin is *light gray*

River (84361 Mg year^{-1}) or the Ohio River (97499 Mg year^{-1}) sub-basins (Table 3). The lower CN_{Out} from the Missouri River sub-basin is likely the result of lower annual precipitation, greater evapotranspiration, and lower stream discharge compared to the other two sub-basins.

Proportional NO_3–N removal (PNR) is strongly influenced by NO_3–N availability ($r = -0.20$ to -0.62, $P < 0.0018$) and stream depth ($r = -0.73$ to -0.93, $P < 0.0001$). Thresholds for PNR are evident for NO_3–N (>2000 μg L^{-1}; Fig. 4a) and depth (>1 m; Fig. 4b), at which point PNR decreased sharply. PNR estimated from the Seitzinger et al. (2002) and Wollheim et al. (2006) models was inversely related to stream order in all three sub-basins. PNR_S and PNR_W were similar (<0.01–0.28) in each of the three sub-basins (Table 3, Fig. 5a). NO_3–N removal (kg km^{-1} d^{-1}) increased with increasing stream order and was greatest in the Upper Mississippi River sub-basin (<0.01–338 kg km^{-1} d^{-1}) followed by the Ohio River (<0.01–243 kg km^{-1} d^{-1}) and Missouri River (<0.01–54 kg km^{-1} d^{-1}; Table 3). Cumulative N removal was usually greatest in the Upper Mississippi River sub-basin (CNR_W 133 Mg year^{-1}; CNR_S 4142 Mg year^{-1}) compared to the Ohio River (CNR_W 378 Mg year^{-1}; CNR_S 3743 Mg year^{-1}) of Missouri River (CNR_W 197 Mg year^{-1}; CNR_S 2277 Mg year^{-1}; Table 3, Fig. 5b).

We found a positive correlation ($r = 0.20$–0.39, $P < 0.0001$) between the percent of the catchment in agricultural uses and NO_3–N concentrations for the Missouri and Ohio Rivers, respectively, but no such correlation for the Upper Mississippi River (Fig. 6a). PNR was negatively correlated ($r = -0.38$ to -0.73, $P < 0.0001$) with percent of the catchment in agriculture in all three sub-basins (Fig. 6b).

Discussion

Not all the N supplied to streams in the Upper Mississippi River basin reaches the Gulf of Mexico.

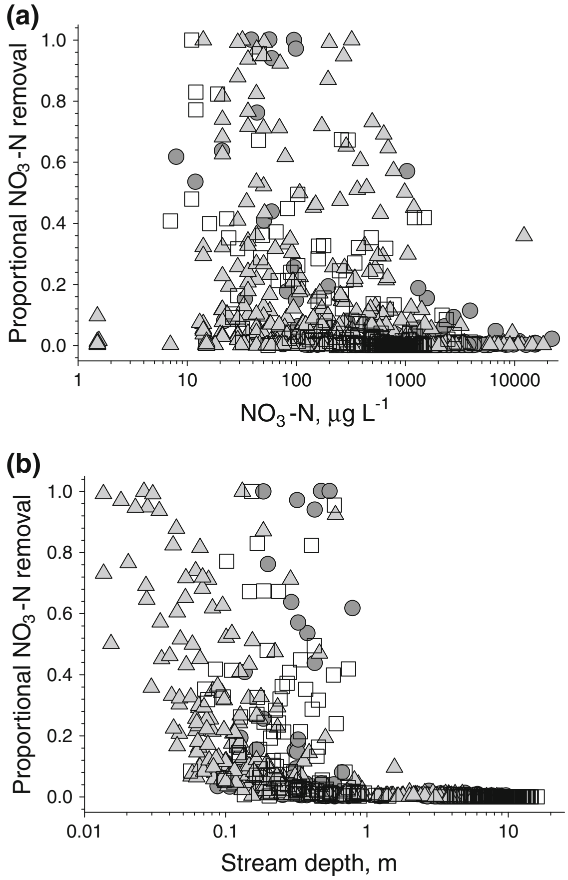

Fig. 4 The relationship between proportional NO_3–N removal (sensu Wollheim et al. 2006) and **a** NO_3–N concentrations ($\mu g\ L^{-1}$) and **b** stream depth (m). The Upper Mississippi River sub-basin streams and rivers are represented by *dark gray circles*; Missouri River sub-basins streams and rivers by *light gray triangles*; and the Ohio River sub-basin streams and rivers by *open squares*

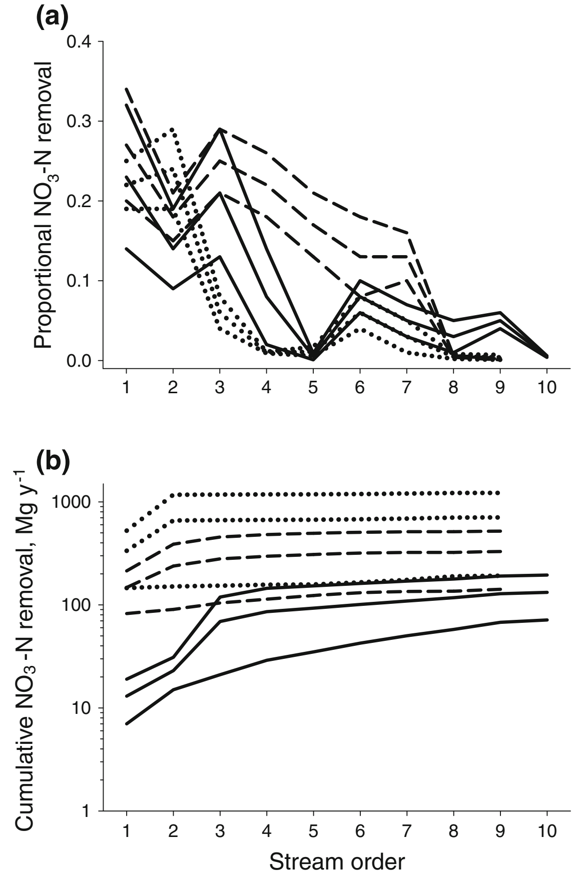

Fig. 5 Mean (±SE) proportional NO_3–N removal (**a**, $kg\ km^{-1}$) and cumulative NO_3–N removal (**b**, $Mg\ year^{-1}$) by stream order for the Upper Mississippi River basin (*solid lines*), Missouri River basin (*dashed lines*), and the Ohio River basin (*dotted lines*). The center line of each triplet of lines is the mean bounded by the upper and lower SE of the mean

Some remains in the streams, indefinitely cycling through biomass. Permanent loss of N results from denitrification in the anoxic sediments. N removal is the net result of interplay between hydrogeomorphic processes that put NO_3–N into contact with biological processes that then create gaseous N_2 and N_2O. Denitrification occurs in all streams but removal efficiency decreases as streams get larger. The extreme diversity in stream size (i.e. depth, width, and discharge), watershed land use (effecting N concentration in runoff), and methods for measuring denitrification make it challenging to develop relationships predicting N removal across large basins (Seitzinger 2008). Survey data for streams and rivers of the Mississippi River basin have the distinct advantages of being statistically representative of all members of the population of streams and rivers by stream order, and using consistent methods for sample collection and analysis. They have the distinct disadvantages of lacking direct measurements of denitrification and one-time, summer sampling. Neither disadvantage could be overcome without incredible expense and effort. Using known relationships between chemical and physical measurements from all the streams and rivers and derived N uptake variables (primarily from streams) was a necessary, but reasonable, compromise.

In the Mississippi River basin (Alexander et al. 2000) and New England streams and rivers (Alexander et al. 2007), the percent removal of N (analogous to our

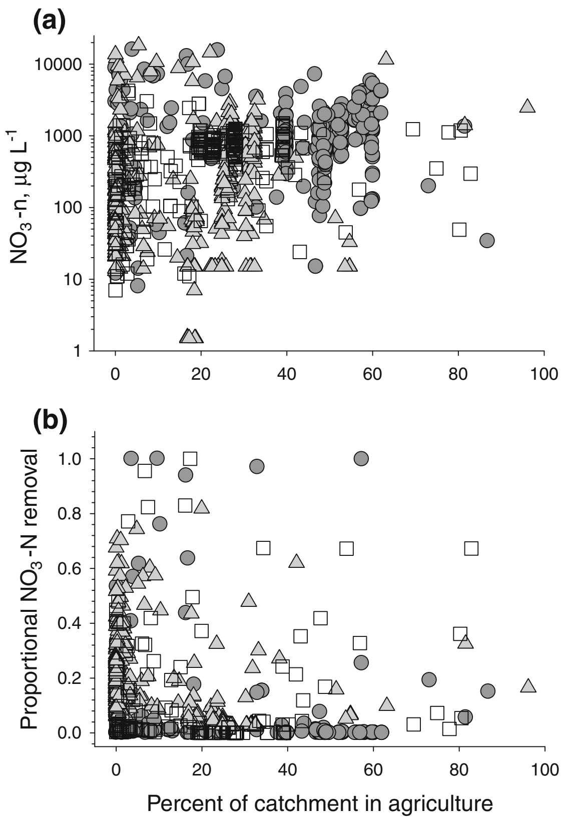

Fig. 6 The relationship between the percent of the catchment in agricultural land uses and **a** NO_3–N concentrations (µg L^{-1}) and **b** proportional NO_3–N removal (sensu Wollheim et al. 2006). The Upper Mississippi River sub-basin streams and rivers are represented by *dark gray circles*; Missouri River sub-basins streams and rivers by *light gray triangles*; and the Ohio River sub-basin streams and rivers by *open squares*

PNR) was inversely related to channel size. Because low-order streams are relatively efficient at N removal and constitute the majority of stream length in the network (1st order streams are approximately 60% of L in all three sub-basins), they are assumed to account for the majority of NO_3–N removal at the basin-scale. Tank et al.'s (2008) "river as a pipe" scenario ascribes the inverse relationship between N removal and stream size to biological uptake in sediments. However, the dominance of headwater or low-order streams in NO_3–N removal in river networks has not been universally corroborated. Using a mass-balance model in 16 drainage basins in northeastern United States, Seitzinger et al. (2002) argued for the importance of larger streams and rivers. They reported that while headwater streams represented 90% of the drainage network channel length they accounted for only half of the total N removed by the network. The remaining half was removed by larger streams and rivers representing only 10% of total channel length. One of the assumptions of the Seitzinger et al. (2002) model was a constant V_f–NO_3 across all stream sizes, analogous to the second scenario proposed by Tank et al. (2008), where biological N demand is constant with increasing stream size. However, the important implication for large rivers is that deep and fast water with restricted floodplain access may not provide sufficient opportunity for NO_3–N to come in contact with benthic stream biota involved in N removal processes. Our survey data reveal that the efficiency of NO_3–N removal from streams in the Mississippi River basin is equally dependent on NO_3–N availability and physical factors that increase the contact of NO_3–N rich waters with sediments.

Our estimates of cumulative NO_3–N removal were made based on the mapped length of streams of all orders (Table 3), the relationship of proportional NO_3–N removal to NO_3–N concentrations and stream depth, and the unbiased sampling of NO_3–N concentrations and stream size. Our cumulative NO_3–N removal estimates were similar to those produced by other N removal models (Ensign and Doyle 2006; Wollheim et al. 2006; Mulholland et al. 2008). We considered net NO_3–N removal rather than estimating assimilation and denitrification separately because NO_3–N in any stream reach is either assimilated into biota, lost via denitrification or dissimilatory reduction of NO_3–N to NH_4–N, or exported downstream (Bernot and Dodds 2005).

We found an increase in NO_3–N concentrations with increasing agricultural land uses in Mississippi River basin catchments, along with a decrease in the proportional NO_3–N removal by streams and rivers draining those catchments (Fig. 6). Hall et al. (2009) found only weak direct connections between N uptake metrics and the proportion of the catchment in agriculture. However, there were significant indirect effects because of strong associations between NO_3–N and the proportion of the catchment in agriculture and between NO_3–N and areal NO_3–N uptake. The potential for agriculture to drive NO_3–N dynamics in the Mississippi River basin was further tested by Donner et al. (2004), who reported that the majority of N runoff was attributable to fertilization of corn and wheat, with a lesser amount coming from N-fixation by soybeans. The greatest N leaching (equivalent to our N input) occurred in the intensively cultivated region of the Mississippi River basin (above the Ohio River confluence). Spatial patterning of N leaching, in particular its proximity to large tributaries, influenced net N output and downstream delivery (Fig. 3). Heavily fertilized catchments relatively close to the main-stem or large tributaries contributed disproportionately to N export to the Gulf of Mexico, a finding supported by models (Alexander et al. 2008). These models are consistent with our results showing that the efficiency of NO_3–N removal declines as stream discharge increases, stream channels get deeper (Fig. 4b), and catchments get larger (Fig. 5a).

In response to the persistent zone of hypoxia in the northern Gulf of Mexico, the Mississippi River/Gulf of Mexico Nutrient Task Force (2001) recommended a 20–40% reduction in NO_3–N exported from the Mississippi–Atchafalaya River basin in order to limit, and possibly reduce, the size of the hypoxia zone. The proposed reductions are to be achieved primarily through reductions in fertilizer applications in the basin resulting in less NO_3–N entering streams and rivers. Given the relationships between NO_3–N concentrations and N uptake rates, reductions in NO_3–N concentrations translate into disproportionally smaller reductions in NO_3–N delivered to the Gulf (Alexander et al. 2007; Mulholland et al. 2008). While our data are from the Upper Mississippi River basin, our NO_3–N load and removal estimates support this hypothesis. Because of the interplay of NO_3–N concentrations and stream size in regulating NO_3–N uptake, a 20% reduction in NO_3–N concentrations would result in a 7–9% increase in proportional NO_3–N removal (PNR), and a 40% reduction in NO_3–N input would result in a 17–23% increase in PNR. Because of the overall low PNR, especially in the Upper Mississippi River sub-basin (Table 3), this increase in PNR has virtually no impact on the export of NO_3–N from the Mississippi River basin.

While many studies have focused on the contribution of headwater streams to N removal (Alexander et al. 2007, 2008; Craig et al. 2008), we found that large rivers, due mostly to a greater width to depth ratio than smaller streams, also removed significant amounts of NO_3–N. The shallower depth relative to width for large rivers overcame the inefficiency of NO_3–N removal and their shorter cumulative lengths within the river network. The net result is that large rivers are comparable to headwater streams as N sinks on the landscape.

Acknowledgements We thank Xiaoli Yuan (USGS Upper Midwest Environmental Sciences Center) for analytical chemistry support for the EMAP samples and the numerous state analytical laboratories for WSA chemistry; Marlys Cappaert and her team (CSC, Corp.) for database support; and Tatiana Nawrocki, Matthew Starry, Roger Meyer, and Jesse Adams (CSC, Corp.) for GIS support. Tony Olsen supervised the creation of the survey designs. We are especially indebted to the field crews who collected the data. The information in this document has been funded wholly by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Alexander RB, Smith RA, Schwartz GE (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758–761

Alexander RB, Boyer EW, Smith RA, Schwarz GE, Moore RB (2007) The role of headwater streams in downstream water quality. J Am Water Res Assoc 43:41–59

Alexander RB, Smith RA, Schwarz GE, Boyer EW, Nolan JV, Brakebill JW (2008) Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Upper Mississippi River basin. Environ Sci Technol 42:822–830

American Public Health Association (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Angradi TR, Bolgrien DW, Jicha TM, Pearson MS, Hill BH, Taylor DL, Schweiger EW, Shepard L, Batterman AR, Moffett MF, Elonen CE, Anderson LE (2009) A bioassessment approach for mid-continent great rivers: the Upper Mississippi, Missouri, and Ohio (USA). Environ Monit Assess 152:425–442

Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442–453

Broussard W, Turner RE (2009) A century of changing land-use and water quality relationships in the continental US. Front Ecol Environ 7:302–307

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89–96

Craig LS, Palmer MA, Richardson DC, Filoso S, Bernhardt ES, Bledsoe BP, Doyle MW, Groffman PM, Hassett BA, Kaushal SS, Mayer PM, Smith SM, Wilcock PR (2008) Stream restoration strategies for reducing river nitrogen loads. Front Ecol Environ 6:529–538

Donner SD, Kucharik CJ, Foley JA (2004) Impact of changing land use practices on nitrate export by the Mississippi River. Global Biogeochem Cycles 18:BG1028-1–21

Earl SR, Valett HM, Webster JR (2006) Nitrogen saturation in stream ecosystems. Ecology 87:3140–3151

Ensign SH, Doyle MW (2006) Nutrient spiraling in streams and river networks. J Geophys Res 111:G04009 1–13
Goolsby DA, Battaglin WA, Lawrence GB, Artz RS, Aulenbach BT, Hooper RP, Keeney DR, Stensland GJ (1999) Flux and sources of nutrients in the Mississippi-Atchafalaya River basin. Topic 3 Report-integrated assessment of hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program Decision Analyses Series no. 17. National Oceanographic and Atmospheric Administration, Silver Springs, Maryland
Hall RO, Tank JL, Sobota DJ, Mulholland PJ, O'Brien JM, Dodds WK, Webster JR, Valett HM, Poole GC, Peterson BJ, Meyer JL, McDowell WH, Johnson SL, Hamilton SK, Grimm NB, Gregory SV, Dahm CN, Cooper LW, Ashkenas LR, Thomas SM, Sheibley RW, Potter JD, Niederlehner BR, Johnson LT, Helton AM, Crenshaw CM, Bergin AJ, Bernot MJ, Beaulieu JJ, Arango CP (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol Oceanogr 54:653–665
Homer C, Huang C, Tang L, Wylie B, Coan M (2004) Development of a 2001 national land-cover database for the United States. Photogramm Eng Remote Sensing 70: 829–840
Howarth RW, Billem G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing JA, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P, Zhao-Liang Z (1996) Regional budgets and riverine N and P fluxes for drainages of the North Atlantic Ocean: natural and human influences. Biogeochemistry 35:75–139
Kaufmann PR, Levine P, Robison EG, Seeliger C, Peck DV (1999) Quantifying physical habitat in wadeable streams. EPA/620/R-99/003. US Environmental Protection Agency, Washington, DC
Mississippi River/Gulf of Mexico Nutrient Task Force (2001) Action plan for reducing, mitigating, and controlling hypoxia in the Northern Gulf of Mexico. US Environmental Protection Agency, Office of Wetlands, Oceans and Watersheds, Washington, DC
Mulholland PJ, Helton AJ, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahn CN, Dodds WK, Findlay SG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulueu JJ, Bernot MJ, Burgin AJ, Crenshaw CJ, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452: 202–206
Olsen AR, Peck DV (2008) Survey design and extent estimates for the Wadeable Streams Assessment. J North Am Benthol Soc 27:822–836
Palmer MA (2009) Reforming watershed restoration: science in need of application and applications in need of science. Estuar Coasts 32:1–17
Rabalais NN (2002) Nitrogen in aquatic ecosystems. Ambio 31:102–112
Seitzinger S (2008) Out of reach. Nature 452:162–163
Seitzinger SP, Styles RV, Boyer EW, Alexander RB, Billen G, Howarth RW, Mayer B, Van Breemen N (2002) Nitrogen retention in rivers: model development and application to watershed in the northeastern USA. Biogeochemistry 57(58):199–237
Strahler AN (1957) Quantitative analysis of watershed geomorphology. Trans Am Geophys Union 38:913–920
US Geological Survey (2001) National land-cover database for the United States, 2001. US Geological Survey, Reston, Virginia. http://www.mrlc.gov. Accessed 10 June 2009
Tank JL, Rosi-Marshall EJ, Baker MA, Hall RO (2008) Are rivers just big streams? A pulse method to quantify nitrogen demand in larger rivers. Ecology 89:2935–2945
Turner RE, Rabalais NN (2003) Linking landscape and water quality in the Mississippi River basin for 200 years. Bioscience 53:563–572
Turner RE, Rabalais NN, Justic D (2008) Gulf of Mexico hypoxia: alternate states and a legacy. Environ Sci Technol 42:2323–2327
US Environmental Protection Agency (2006) Wadeable streams assessment: a collaborative study of the Nation's streams. EPA 841-B05-002. US Environmental Protection Agency, Office of Water, Washington, DC
Wollheim WM, Vorosmarty CJ, Peterson BJ, Seitzinger SP, Hopkinson CS (2006) Relationship between river size and nutrient removal. Geophys Res Lett 33:L06410