Biogeochemistry (2010) 97:195–209
DOI 10.1007/s10533-009-9366-0

Sediment microbial enzyme activity as an indicator of nutrient limitation in the great rivers of the Upper Mississippi River basin

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Received: 10 December 2008 / Accepted: 13 August 2009 / Published online: 4 September 2009
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Abstract We compared extracellular enzyme activity (EEA) of microbial assemblages in river sediments at 447 sites along the Upper Mississippi, Missouri, and Ohio Rivers with sediment and water chemistry, atmospheric deposition of nitrogen and sulfate, and catchment land uses. The sites represented five unique river reaches—impounded and unimpounded reaches of the Upper Mississippi River, the upper and lower reaches of the Missouri River, and the entire Ohio River. Land use and river chemistry varied significantly between rivers and reaches. There was more agriculture in the two Upper Mississippi River reaches, and this was reflected in higher nutrient concentrations at sites in these reaches. EEA was highest in the two Upper Mississippi River reaches, followed by the lower Missouri River reach. EEA was generally lowest in the upper Missouri River reach. Canonical correlation analysis revealed a strong correlation between EEA and the suite of water and sediment chemistry variables, and the percent of the catchment in anthropogenically dominated land uses, including agriculture and urban development. Nutrient ratios of the waters and sediments suggested carbon (C), nitrogen (N), or phosphorus (P) limitation at a large number of sites in each reach. C-limitation was most pronounced in the unimpounded Mississippi River and lower Missouri River reaches; N-limitation was prevalent in the two Missouri River reaches; and P-limitation dominated the Ohio River. Linking microbial enzyme activities to regional-scale anthropogenic stressors in these large river ecosystems suggests that microbial enzyme regulation of carbon and nutrient dynamics may be sensitive indicators of anthropogenic nutrient and carbon loading.

Keywords Microbial enzymes · Nutrients · Stoichiometry · Upper Mississippi River basin

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Introduction

In most aquatic ecosystems a significant portion of energy and nutrient flow is through the microbial loop. Extracellular enzymes are produced by the microbial assemblage to aid in the degradation of organic matter and the resultant acquisition of limiting nutrients (Sinsabaugh and Foreman 2001). Biofilm extracellular enzyme activity (EEA) is integrated into broader energy and nutrient cycling processes, and is therefore difficult to measure directly. To overcome this problem, researchers have turned to the use of labelled organic compounds that have characteristics similar to the naturally occurring substances for which they are

analogs, and have a fluorochrome which is activated by the enzymatic cleavage of specific glycoside, peptide, or ester bonds. Detection limits of these fluorogenic compounds are in the nanomolar range (Hoppe 1991). The glycosidases are linked to organic carbon (C) processing; the peptidases are linked to protein degradation and nitrogen (N) cycling; the esterases are linked to phosphorus (P) and sulfur (S) acquisition. Therefore, we measure the activities of these enzymes by proxy with the labeled enzyme-specific compounds. The stoichiometry of these enzyme activities then become indicators of the relative nutrient limitations on microbial assemblages.

Organic C sequestration (productivity) by microbial assemblages is governed not only by organic C availability, but also by the availability of N and P. The ratio of these components in planktonic algae, for example, is expected to be 108C:16N:1P (Redfield 1958; Falkowski 2000) and departures from this ratio in the waters the plankton inhabit suggest that plankton growth will be limited by the element in short supply. While it is generally accepted that freshwater ecosystems in the northern hemisphere are P-limited (Hutchinson 1971; Turner et al. 2003), there is mounting evidence that, based on Redfield ratios of nutrient concentrations, N-limitation may be more common than previously suspected (Bedford et al. 1999; Falkowski 2000). This N-limitation of primary producers may be exacerbated in freshwater rivers, where sediment anoxia frequently precludes using O_2 as the electron acceptor, and anaerobic oxidation of organic C may be limited by the availability of NO_3^- as an electron acceptor, and/or because P enrichment by eutrophication tends to make P relatively more available (Capone and Kiene 1988; Sundareshwar et al. 2003).

Respiration in aquatic systems is usually measured as O_2 consumption, but it may also be measured as electron transport system activity using relative levels of dehydrogenase enzymes (Broberg 1985). Dehydrogenase activity (DHA) measures the activity of the oxidation–reduction enzymes of cellular electron transport system (Packard 1971; Broberg 1985). It has been used to measure the activity of stream microbial communities and their responses to disturbances (Trevors et al. 1982; Blenkinsopp and Lock 1990).

River ecosystems around the world are impacted by catchment developments for human uses. These developments, including agriculture, urbanization, industrial and metropolitan effluents, and channel modifications, are occurring at an unprecedented rate, and the resulting alteration, impairment, and destruction of river ecosystems is greater than at any other time in human history. Nutrient and sediment influx to rivers, as nonpoint source pollution, has become the leading source of water quality degradation in rivers of the United States (Baker 1985; Turner and Rabalais 2003). The linkage between surface water nutrient concentrations and sediment nutrient content suggests the possibility of monitoring water quality status and trends using sediment microbial properties, such as EEA, that integrate conditions through time as surrogates for water and sediment chemistry.

The purpose of this research was to compare the extracellular enzyme activity (EEA) of sediment microbial assemblages in their processing of organic C with nutrient chemistry in rivers of the Upper Mississippi River basin (UMRB), and to evaluate the use of EEA as an indicator of nutrient enrichment and/or limitation in these rivers. We compared a suite of enzymes produced by sediment microbial assemblages for the acquisition of organic carbon with measured nutrients in those sediments and the overlying waters in the Missouri, Upper Mississippi, and Ohio Rivers. Our underlying premise is that organic matter processing by sediment microbial assemblages is so tightly governed by C:N:P ratios that carbon processing rates will be directly controlled by nutrient availability and with the land uses that govern nutrient inputs to the rivers. We hypothesize that EEA will directly reflect not only the activity of the microbial assemblage, but also the nutrient status of the environment. Hence, as nutrient concentrations increase, we expect a corresponding shift in the allocation of microbial enzymes toward those devoted to C acquisition relative to those involved in N and P acquisition.

Methods

Site selection

The US Environmental Protection Agency's (US EPA) Environmental Monitoring and Assessment Program for Great Rivers (EMAP-GRE) includes the Upper Mississippi River from Lower St. Anthony Falls in Minneapolis-St. Paul, Minnesota to the

confluence with the Ohio River at Cairo, IL; 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. The six reservoirs on the Missouri River in North and South Dakota were excluded from the program, because their long hydrologic turnover times (>1 year, National Research Council 2002) made them more lentic than lotic systems. The EMAP-GRE design was spatially balanced and employed an unequal probability for selecting sites based on unique river reaches, e.g., the unimpounded Upper Mississippi River. The survey design selected a single point on the river center line as defined by the National Hydrography Database (NHD). All sampling at a site was done in relation to that point, defined by latitude and longitude coordinates, and all site data related to this point for population estimates (Angradi 2006; Angradi et al. 2009). The resulting design selected 447 sites representing 4,838 km of the main channel of the UMRB (Fig. 1). Approximately 20% of the sites were re-sampled for quality assurance purposes resulting in a total of 533 site-visits. Sampling at these sites occurred July–September 2004–2006. The sites and visits were apportioned among the following unique river reaches: the impounded Upper Mississippi River (upstream of the confluence with the Missouri River, 122 sites, 144 site-visits); the unimpounded Upper Mississippi River (downstream of the confluence with the Missouri River, 22 sites, 29 site-visits); the upper Missouri River (53 sites, 57 site-visits in the Ft. Peck, Garrison, and Missouri National Recreational River reaches, Fig. 1); the lower Missouri River (130 sites, 156 site-visits); and the entire Ohio River (120 sites, 146 site-visits).

Sediment collection and size fractionation

Sediment samples at each site were collected at 11 stations equally spaced along a 500 m, longitudinal channel-margin transect. We chose to focus on surface sediments because of their direct contact with the overlying river waters. We collected surface sediments (top 5 cm) using a scoop or dredge. Sediments were combined for all stations at a site, resulting in a single sample per site. Samples were stored on ice, and returned to the laboratory for processing. Sediment size fractions were determined gravimetrically for each sediment sample by wet sieving (Guy 1969). Sediments at each sites were characterized as being dominated by gravel (>2,000 μm diameter), coarse sand (250–2,000 μm), fine sand (63–250 μm), and silts and clays (<63 μm) based on percent representation of these classes in the composite sample.

Water chemistry

Water samples were collected as cross-channel depth-integrated composites. Samples were homogenized in a churn splitter and shipped to the lab on ice within 36 h of collections. We consider only total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC), and sulfate (SO_4^{-2}) in this paper, but see Angradi (2006) for a full list of analytes. All nutrient samples were analyzed using a Bran-Lubbe continuous flow analyzer (Model AA3, SEAL Analytical Inc., Mequon, WI, USA) with appropriate methods. Unfiltered subsamples were digested (persulfate method) and analyzed for TN (cadmium reduction method) and TP (molybdate-ascorbic acid method, APHA 1998). Samples for TOC were preserved with phosphoric acid, stored at 4°C in amber glass containers, and then analyzed by UV promoted, persulfate oxidation on a Tekmar–Dohrmann organic carbon analyzer (Model Phoenix 8000, Teledyne Tekmar, Mason, OH, USA, APHA 1998). Samples for SO_4^{-2} were immediately frozen and stored (<28 days) until analyzed by Dionex ion chromatograph (Model ICS 2000, Dionex Corporation, Sunnyvale, CA, USA, APHA 1998).

Sediment chemistry

Soil water content was determined gravimetrically and percent solids were used to calculate available nutrient content on a dry weight basis. Sediment samples were dried and ground for nutrient analysis. Total N and C were determined by the combustion method using a Carla Erba elemental analyzer (Model 1112EA, Carla Erba Instrumentazione, Milan, Italy). Total P was determined by first digesting the sample in reagent grade concentrated HNO_3 using an Anton Parr Multiwave microwave (Anton Paar GmbH, Graz, Austria). Samples were then diluted to 90 ml with deionized water, neutralized with 10 ml NaOH, and

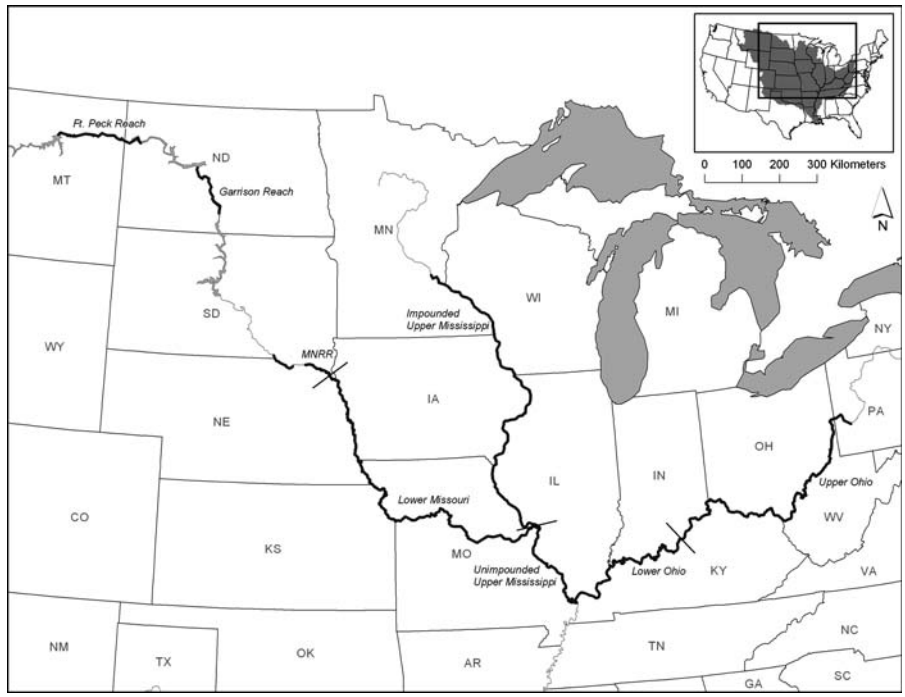

Fig. 1 Locations of the reaches of the Upper Mississippi River Basin. The Ft. Peck, Garrison, and Missouri National Recreational River (MNRR) reaches were combined into a single Upper Missouri River reach. Sampled reaches are indicated by *black river traces*; unsampled reaches are indicated by *gray river traces*

analyzed by the molybdate-ascorbic acid method (APHA 1998).

Land cover

Land cover data corresponding with the NHD-defined catchment for each site were taken 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 United States. Land covers were aggregated into five classes for analyses: agriculture, developed, 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 to 77%, 83 to 91%, and 78 to 93%, respectively (Homer et al. 2004).

Atmospheric deposition

Data for atmospheric deposition of TN, SO_4^{-2}, and precipitation were available from the National Atmospheric Deposition Program (NADP, http://nadp.sws.uiuc.edu). We used annual (2001–2006) precipitation-weighted mean TN and SO_4^{-2} concentrations in precipitation. Estimates of TN and SO_4^{-2} deposition and precipitation at each of our study sites were based on NADP station data that were intersected with spatially-interpolated national grids (http://nadp.sws.uiuc.edu/isopleths). These interpolated data were averaged across the years of our study.

Sediment microbial enzyme activity

In the laboratory, sediments collected from each stream transect were thawed, mixed, and analyzed for dehydrogenase activity (DHA) and extracellular enzyme activity (EEA). Duplicate DHA aliquots were mixed with 2 ml of sterile H_2O and 1 ml of 0.75% 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) standard, sealed, agitated for 1 min with a vortex mixer, and incubated (dark, 27°C) for 3 h. Duplicate analyses without a killed control were deemed acceptable based on our previous experience (Hill et al. 2002) that indicated variance among replicates was much less than the

variance among sites. Among site variance for this study is 5–10%, compared to <5% within site variance.

Incubations were terminated by adding 8 ml of methanol. Aliquots were centrifuged (2000*g*) for 5 min and the supernatant analyzed for absorbance (428 nm) using a Perkin Elmer UV (Model Lambda 20) spectrophotometer (Perkin Elmer Life And Analytical Sciences, Inc., Wellesley, MA, USA). Aliquot absorbances were compared to a standard INT curve (prepared for each sample batch) and normalized by dry weight (Carla Erba Model 1112EA) to calculate DHA activity (nmol g^{-1} DW h^{-1}).

We analyzed sediment samples for six glycosidases (α-D-galactosidase [EC 3.2.1.22], β-D-galactosidase [EC 3.2.1.23], α-D-glucosidase [EC 3.2.1.20], β-D-glucosidase [EC 3.2.1.21], β-*N*-acetylglucosaminidase [EC 3.2.1.50], β-D-xylosidase [EC 3.2.1.8]); four aminopeptidases (L-alanine [EC 3.4.11.2], L-arginine aminopeptidase [EC 3.4.11.x], L-glycine aminopeptidase [EC 3.4.11.x], L-leucine aminopeptidase [EC 3.4.11.1]); and two esterases (phosphatase [EC 3.1.3.2], sulfatase [EC 3.1.6.1]), using substrates linked to methylumbelliferyl (MUB) or coumarin (MCM) residues (Sigma–Aldrich Corp., St. Louis, MO, USA), and the microplate protocols developed by Sinsabaugh and colleagues (Sinsabaugh et al. 1997; Foreman et al. 1998; Sinsabaugh and Foreman 2001). Each microplate array included quadruplicate assays for each enzyme and each reference standard. All substrate and reference solutions were prepared in sterile deionized water. Quenching, the decrease of fluorescent emissions caused by the interactions of enzyme substrates with non-reactant chemicals in the assays, was estimated by comparing the fluorescence of the supernatant of standard solutions mixed with river sediments with that of the standard solution mixed only with buffer. Substrate and sample controls (each mixed with buffer) were assayed in quadruplicate on the same microplate. Microplates were incubated in the dark at 30°C, similar to the median temperature (26°C) for the rivers. Fluorescence was measured at 60 min intervals using a BioTek (Model FLX800T) fluorometer (BioTek Instruments, Winooski, VT, USA) with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. We report EEA as substrate accumulated over time (nmol g^{-1} DW h^{-1}) adjusted for emission coefficients calculated from standards, and corrected for quenching.

Statistical analyses

The relationships between the EEA variables and the environmental variables were evaluated using Spearman rank correlation (*r*s) to avoid problems associated with non-normal data distribution.

Environmental variables significantly correlated with EEA were selected for subsequent investigation using canonical correlation analysis (CCA). CCA computes a series of canonical functions that do the best job possible of summarizing the relationship between a linear combination of dependent (diatom index and metrics) variables and a linear combination of independent (environmental) variables. Where two or more variables were highly correlated ($r > 0.5$. e.g. NO_3^- and total N), only the variable with the greatest correlation with the biotic variables was included in CCA. The significance of the canonical correlations ($P < 0.05$) was tested using a *t*-test of the null hypothesis that $r_k = 0$, $t = rk/\sqrt{(1 - rk^2)/(n - m)}$where r_k is the canonical correlation coefficient, n the sample size and m the number of variables (Rohlf and Sokal 1969). All statistical analyses were done using the SAS System for Windows 9 statistical software (SAS Institute, Inc. Cary, NC, USA).

Results

Catchment land use and atmospheric deposition

Agricultural land uses, including pastures and hay fields, were greatest in the catchments of the impounded and unimpounded reaches of the Upper Mississippi River compared to the other UMRB reaches, with means of 52 and 40% of the catchment area for the impounded and unimpounded reaches, respectively (Table 1). Mean forested land cover was 60% in the Ohio River; mean wetland cover ranged from <1% in the Ohio River to 9% in the impounded Mississippi River; mean natural land cover was greatest in the Missouri River reaches (74 and 71%, respectively), and urban development was greatest in the Ohio River (10%) and in the impounded Mississippi River reach (7%, Table 1). Atmospheric TN deposition on the catchments was highest in the Ohio River (58 kg km^{-2} $year^{-1}$) and lowest in the upper

Table 1 Mean (±SE) catchment land use (% of catchment), atmospheric total N and SO_4^{-2} deposition (kg km^{-2} $year^{-1}$), water chemistry (TN, TP, μg l^{-1}; TOC, SO_4^{-2}, mg l^{-1}) and stoichiometry, sediment chemistry (mg kg^{-1}) and stoichiometry, and sediment size fractions (%) for sites in the impounded (MS IMP) and unimpounded (MS UN) Mississippi River, the upper (MO UP) and lower (MO LO) Missouri River, and the Ohio River (OH) reaches

Attribute, units	MS IMP	MS UN	MO UP	MO LO	OH
Catchment land use					
Agriculture	52 (<1)	40 (<1)	24 (1)	27 (<1)	27 (<1)
Developed	7 (<1)	4 (<1)	2 (<1)	2 (<1)	10 (<1)
Forested	28 (<1)	12 (<1)	11 (<1)	8 (<1)	60 (<1)
Natural	41 (<1)	56 (<1)	74 (2)	71 (<1)	64 (<1)
Wetlands	9 (<1)	3 (<1)	1 (<1)	2 (<1)	<1 (<1)
Atmospheric deposition					
TN	55 (0.3)	55 (0.8)	20 (0.7)	52 (0.2)	58 (0.2)
SO_4^{-2}	107 (1)	169 (3)	238 (4)	921 (2)	212 (2)
Water chemistry					
TN	2156 (87.5)	1828 (144)	250 (33.3)	1245 (66.5)	1152 (21.0)
TP	168 (4.01)	249 (10.2)	43.5 (6.75)	219 (13.2)	52.94 (2.41)
SO_4^{-2}	38.8 (0.93)	75.6 (2.64)	135 (2.92)	167 (2.72)	77.2 (2.11)
TOC	7.07 (0.13)	4.55 (0.16)	2.58 (0.06)	3.49 (0.07)	3.08 (0.04)
N:P	44.8 (13.7)	16.6 (1.27)	25.6 (5.76)	18.3 (2.30)	58.0 (1.94)
C:N	4.59 (0.18)	3.22 (0.19)	18.8 (3.04)	5.86 (0.62)	3.27 (0.08)
C:P	177 (58.5)	48.8 (2.33)	394 (80.8)	155 (44.0)	192 (8.20)
Sediment chemistry and size fractions					
Sed C	11716 (690)	7486 (1126)	7543 (494)	9796 (442)	14927 (643)
Sed N	8697 (1077)	4437 (1138)	5179 (717)	10607 (4144)	8512 (1290)
Sed P	38.4 (1.96)	21.8 (3.48)	3.84 (0.33)	23.7 (1.30)	17.1 (0.63)
Sed N:Sed P	27.6 (3.53)	14.6 (3.39)	14.5 (2.11)	26.7 (9.83)	47.4 (19.8)
Sed C:Sed N	84.2 (13.9)	86.8 (31.0)	156 (31.6)	143 (18.6)	196 (28.2)
Sed C:Sed P	43.4 (1.85)	28.9 (2.96)	24.3 (1.41)	30.9 (1.23)	75.2 (22.6)
Gravel	7.78 (1.98)	4.51 (2.06)	0.40 (0.21)	4.71 (1.01)	4.90 (2.03)
Coarse sand	25.4 (3.80)	29.6 (12.7)	25.2 (6.02)	9.40 (1.62)	13.7 (2.27)
Fine sand, %	33.9 (3.25)	33.9 (7.97)	50.7 (4.33)	41.1 (1.93)	52.7 (2.79)
Silt & clay, %	35.5 (3.89)	36.6 (11.5)	26.1 (4.98)	46.8 (2.89)	35.4 (2.68)

Missouri River reach (20 kg km^{-2} $year^{-1}$). This reflects the remoteness of the upper Missouri River in contrast to the more industrialized eastern portions of the UMRB; this trend is not upheld by atmospheric SO_4^{-2} deposition, which is highest in the Upper Missouri reach and lowest in the impounded Mississippi reach (Table 1).

Water quality

Water chemistry varied significantly between the five reaches of the UMRB. TN concentrations were highest in the impounded and unimpounded Mississippi River reaches (1,828 and 2,156 μg l^{-1}) and lowest in the upper Missouri reach (250 μg l^{-1}, Table 1). TN was correlated with percent of the catchment in agriculture, natural land use, and wetlands (Table 2). TP was lowest in the upper Missouri (43.5 μg l^{-1}) and highest in the unimpounded Mississippi reach (249 μg l^{-1}, Table 1) and correlated with the percent of the catchment in wetlands, agriculture and forests (Table 2). SO_4^{-2}, which was highest in the two Missouri reaches (135 and 167 mg l^{-1}, respectively) and lowest in the impounded Mississippi reach

(38.8 mg l^{-1}), was correlated with the percent of the catchment in natural land uses, agriculture, and development (Tables 1 and 2). TOC was highest in the impounded Mississippi reach (7.07 mg l^{-1}), lowest in the upper Missouri reach (2.58 mg l^{-1}, Table 1) and correlated with percent of the catchment in wetlands, agriculture, and natural land uses (Table 2).

The molar ratio of TN and TP were close to the 16:1 Redfield ratio in the unimpounded Mississippi (16.6:1) and lower Missouri (18.3:1) reaches, suggesting no N or P limitation. The remaining reaches had N:P greater than 24:1 suggesting increasing degrees of P-limitation (Table 1). Ratios of TOC, TN and TP also indicated varying degrees of C, N, and P-limitation compared with Redfield's suggested C:N of 7:1 and C:P of 108:1 (Table 1). Using conservative bounds (±50% of the Redfield ratio), the extent of C, N, P-limitation in the five reaches of the UMRB can be assessed. Overall, C-limitation was restricted to the unimpounded Mississippi (86% of the reach) and lower Missouri (65%) reaches, and probably reflects the high N concentrations of the waters relative to the C concentrations. Only the upper Missouri reach exhibited appreciable N-limitation (32% of the reach); P-limitation was evident in the Ohio River (98%), the impounded Mississippi reach (57%), and the upper Missouri reach (28%) (Table 3, Fig. 2).

Sediment chemistry

Unlike nutrient concentrations in the water, there was considerable overlap in the sediment N and P content among the five reaches of the UMRB (Table 1). Average sediment TN ranged from 4,437 mg kg^{-1} in the unimpounded Mississippi River to 10,607 mg kg^{-1} in the lower Missouri River, exhibited high within-reach variability (Table 1), and was not significantly correlated with any of the land use or atmospheric deposition variables (Table 2). Sediment TP was highest in the impounded Mississippi River reach (38.4 mg kg^{-1}), lowest in the upper Missouri River reach (3.84 mg kg^{-1}), and was correlated with the % of the catchment in agriculture, natural land uses, and wetlands (Table 2). Total sediment C varied considerably among the UMRB reaches, ranging 14,927 mg kg^{-1} in the Ohio River to 7,486 mg kg^{-1} in the unimpounded Mississippi reach (Table 1), and was correlated with the percent of the catchment in development and in forests, and with atmospheric SO_4^{-2} TN deposition (Table 2).

The molar ratios of sediment N and P were near the 16:1 Redfield ratio in the upper Missouri River (14.5:1) and unimpounded Mississippi River reaches (14.6:1) and above the Redfield ratio in the remaining reaches (26.7–47.4:1) indicating increasing P-limitation (Table 1). Applying Redfield's ratios

Table 2 The three strongest land use and atmospheric deposition correlates (Spearman *r*) with water chemistry, sediment chemistry, and sediment size fractions in the Upper Mississippi River basin

Variable	Correlate, *r*		
Water chemistry			
TN	% Agriculture, 0.64	% Natural, −0.64	% Wetland, 0.46
TP	% Wetland, 0.61	% Agriculture, 0.52	% Forest, −0.48
SO_4^{-2}	% Natural, 0.82	% Agriculture, −0.68	% Developed, −0.58
TOC	% Wetland, 0.75	% Agriculture, 0.69	% Natural, −0.65
Sediment chemistry and size fractions			
Sed N	ns	ns	ns
Sed P	% Agriculture, 0.56	% Natural, −0.51	% Wetland, 0.38
Sed C	% Developed, 0.39	Atmos.SO_4^{-2} dep., 0.38	% Forest, 0.35
% Gravel	% Wetland, 0.16	ns	ns
% Coarse sand	% Natural, −0.20	% Forest, 0.17	ns
% Fine sand	% Agriculture, −0.36	% Wetland, −0.34	% Natural, 0.29
% Silt & clay	% Forest, −0.19	ns	ns

The significance value for all of these rank correlations is $p < 0.01$ (ns indicates that no variable met this criterion)

Table 3 Percent of the impounded (MS IMP) and unimpounded (MS UN) Mississippi River, the upper (MO UP) and lower (MO LO) Missouri River, and the Ohio River (OH) reaches

Limited by C, N, or P availability determined by water and sediment chemistry and by enzyme activity. Values in parentheses are N or P limitation without considering C-limitation. See text for explanations of C:N:P expectations and thresholds

Limitation	MS IMP	MS UN	MO UP	MO LO	OH
Water					
C-limited	2	86	2	65	1
N-limited	0 (0)	0 (0)	32 (32)	1 (9)	0 (0)
P-limited	57 (58)	3 (17)	28 (29)	6 (6)	98 (98)
Not limited	41 (42)	10 (83)	38 (39)	28 (85)	1 (2)
Sediment					
C-limited	64	59	60	60	100
N-limited	34 (44)	41 (55)	40 (49)	40 (49)	0 (60)
P-limited	1 (48)	0 (38)	0 (33)	0 (40)	0 (40)
Not limited	1 (8)	0 (7)	0 (18)	0 (11)	0 (0)
Enzymes					
C-limited	74	86	64	64	79
N-limited	0 (12)	0 (14)	0 (0)	0 (4)	1 (3)
P-limited	26 (88)	14 (86)	36 (100)	36 (96)	20 (97)
Not limited	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

(108C:7N:16 P) to our data resulted in 59–100% of the sites in all UMRB reaches being C-limited, 0-41% were N-limited, and few sites were P-limited (Table 3). If C-limitation is ignored, then the proportion of sites experiencing N or P-limitation rises significantly (Table 3, Fig. 2).

Sediment size fractions did not vary appreciably among the five UMRB reaches. River sediments were dominated by fine sand (34–52%) followed by silt and clay (26–47%), coarse sand (9–30%), and gravel (<1–8%) (Table 1). The percent gravel was negatively correlated with the percent of the catchment in agriculture; % coarse sand was correlated with the percent of the catchment in natural and forest land uses; % fine sand was negatively correlated with the percent of the catchment in agriculture and wetlands, and positively correlated with the percent of the catchment in natural land uses; and % silt and clay was inversely correlated with percent of the catchment in forests (Table 2). None of the other sediment size classes were correlated with land use or atmospheric deposition variables (Table 2).

Canonical environmental gradients

Canonical correlation analyses (CCA) revealed two significant environmental gradients. The first gradient (W_1) was positively correlated with water and sediment chemistry, and with percent silt and clay sediments. The second canonical gradient (W_2) was

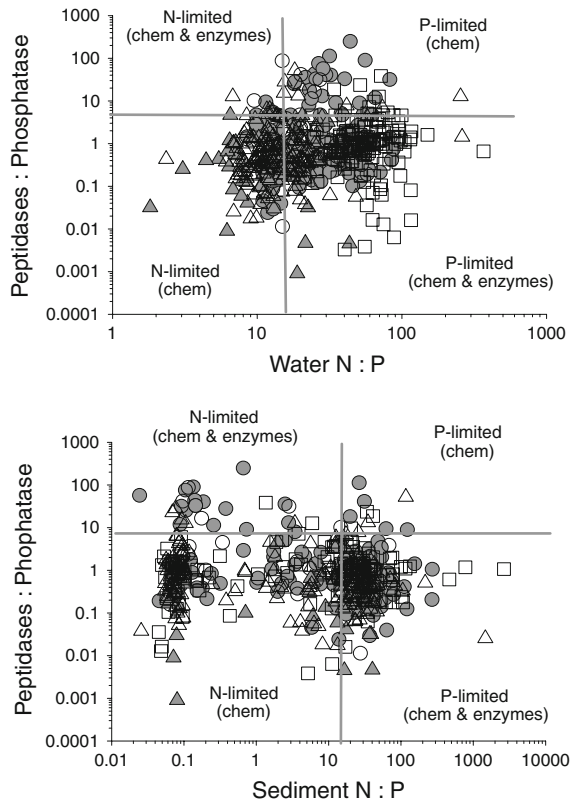

Fig. 2 Scatter plots of water column (*top panel*) and sediment (*bottom panel*) N:P versus microbial peptidases:phosphatase in the impounded Mississippi River (*shaded circles*), the unimpounded Mississippi River (*open circles*), the upper Missouri River (*shaded triangles*), lower Missouri River (*open triangles*), and the Ohio River (*open squares*)

negatively correlated with river water SO_4^{-2} concentrations and positively correlated with the TN, TOC and sediment P, with catchment land uses, and with atmospheric deposition of TN and SO_4^{-2} (Table 4). Overall, these two gradients extracted 69 and 19% of the variance in the correlations of EEA with the environmental variables (Table 4). Several environmental variables, including river O_2 concentrations, temperature, and pH, were rejected for inclusion in the CCA because of insignificant correlations with the enzyme variables.

Extracellular enzyme activity

There was little difference in DHA, an indicator of overall microbial respiratory activity, in the five reaches of the UMRB. Dehydrogenase activity ranged from 616 nmol g^{-1} DW h^{-1} in the impounded Mississippi River reach to 402 nmol g^{-1} DW h^{-1} in the unimpounded Mississippi River reach (Table 5). DHA was correlated with the first canonical environmental gradient (Table 4, Fig. 3a).

Table 4 Correlations of Upper Mississippi River EEA measures with the canonical environmental gradients (W_1, W_2)

Variable	W_1	W_2
Water chemistry		
TN	**0.33**	**0.71**
TP	**0.39**	0.31
TOC	0.22	**0.78**
SO_4^{-2}	−0.14	**−0.87**
Sediment chemistry and size fractions		
Sed C	**0.85**	−0.10
Sed N	**0.40**	0.19
Sed P	**0.48**	**0.38**
% Silt & clay	**0.77**	−0.30
Catchment land use		
% Agriculture	0.15	**0.78**
% Developed	0.13	**0.67**
% Wetlands	0.09	**0.71**
Atmospheric deposition		
TN	0.28	**0.52**
SO_4^{-2}	0.28	**0.53**
Microbial enzymes		
DHA	**0.31**	−0.06
GLYC	**0.79**	0.09
PEPT	0.21	**0.44**
PHOS	**0.68**	−0.01
SULF	**0.63**	0.16
Total variance extracted (%)	69	19

Significant correlations ($p < 0.05$) are in bold

The glycosidases, indicators of microbial C acquisition, exhibited considerably more variability between river reaches than DHA, being highest in the impounded Mississippi River reach and lowest in the upper Missouri River reach (Table 5). Overall, β-D-glucosidase had the highest activity among the glycosidases, ranging from 47 nmol g^{-1} DW h^{-1} in the upper Missouri River reach to 125 nmol g^{-1} DW h^{-1} in the impounded Mississippi River reach (Table 5). This was followed by β-*N*-acetylglucosaminidase activity (25.5–60.4 nmol g^{-1} DW h^{-1}) (Table 5), and the remaining glycosidases (α-D-galactosidase, β-D-galactosidase, α-glucosidase, and β-D-xylosidase) (Table 5). Total glycosidase activity (GLYC) ranged from 88.5 nmol g^{-1} DW h^{-1} in the upper Missouri River reach to 233 nmol g^{-1} DW h^{-1} in the impounded Mississippi River reach (Table 5). GLYC was correlated with the first canonical environmental gradient (Table 4, Fig. 3b).

As with the glycosidases, aminopeptidase activities, an indicator of microbial N acquisition, were highest in the impounded Mississippi River reach and lowest in the upper Missouri River reach (Table 5). L-leucine and L-alanine aminopeptidases dominated, with activities ranging from 12.4 to 197 nmol g^{-1} DW h^{-1} and 11.3 to 199 nmol g^{-1} DW h^{-1}, respectively (Table 4). L-arginine and L-glycine aminopeptidase activities ranged from 39.2 to 59.9 nmol g^{-1} DW h^{-1} and 46.9 to 74.4 nmol g^{-1} DW h^{-1} (Table 5). Total aminopeptidase activity (PEPT) was correlated with the second canonical environmental gradient (Table 4, Fig. 3c).

Despite significant differences in water and sediment P concentrations between UMRB reaches, phosphatase activity, an indicator of microbial P acquisition, exhibited no significant differences between these reaches (Table 5). Phosphatase activity (PHOS) ranged from 71.7 to 123 nmol g^{-1} DW h^{-1}, and was slightly higher in the P-limited Ohio River. PHOS was correlated with the first canonical environmental gradient (Table 4, Fig. 3d).

Sulfatase activity (SULF), an indicator of microbial S acquisition, was highest in the impounded Mississippi River reach (25.7 nmol g^{-1} DW h^{-1}) and lowest in the upper Missouri River reach

Table 5 Mean (±SE) microbial dehydrogenase and extracellular enzyme activities (nmol g^{-1} DW h^{-1}), and stoichiometric ratios of glycosidases (GLY), peptidases (PEP), and Phosphatase (PHO) for sites in the impounded (MS IMP) and unimpounded (MS UN) Mississippi River, the upper (MO UP) and lower (MO LO) Missouri River, and the Ohio River (OH) reaches

	MS IMP	MS UN	MO UP	MO LO	OH
Dehydrogenase	1,307 (76.6)	853 (91.0)	1,135 (176)	1,103 (196)	1,090 (115)
α-D-Galactosidase	11.3 (0.94)	7.05 (1.43)	4.50 (0.47)	8.38 (0.68)	6.08 (0.37)
β-D-Galactosidase	14.9 (1.10)	10.7 (3.70)	4.14 (0.52)	9.06 (0.74)	10.5 (0.84)
α-D-Glucosidase	12.3 (1.34)	11.2 (6.14)	2.79 (0.51)	6.95 (1.29)	3.11 (0.28)
β-D-Glucosidase	125 (8.58)	92.3 (23.9)	47.0 (5.33)	107 (6.43)	95.3 (6.56)
β-*N*-Acetylglucosaminidase	60.4 (4.53)	31.6 (6.73)	25.5 (2.63)	50.7 (4.65)	54.8 (3.30)
β-D-Xylosidase	9.58 (0.62)	6.08 (0.99)	4.57 (0.34)	7.21 (0.42)	5.81 (0.36)
Total glycosidases	233 (13.4)	159 (35.1)	88.5 (8.43)	189 (10.6)	176 (10.6)
L-Alanine	199 (36.2)	127 (53.4)	11.3 (3.52)	94.8 (24.0)	30.6 (3.93)
L-Arginine	59.9 (93.64)	55.2 (5.29)	39.2 (3.63)	46.8 (2.32)	45.9 (1.97)
L-Glycine	74.4 (3.97)	68.1 (6.46)	46.9 (4.42)	59.0 (3.52)	54.6 (2.39)
L-Leucine	197 (37.7)	151 (62.2)	12.4 (4.82)	81.2 (23.7)	24.4 (3.91)
Total aminopeptidases	396 (73.6)	278 (112)	23.7 (7.83)	176 (47.3)	55.0 (7.79)
Phosphatase	83.9 (6.81)	71.7 (22.1)	90.2 (51.6)	83.4 (13.0)	123 (34.2)
Sulfatase	25.7 (2.63)	10.3 (2.89)	6.48 (1.18)	11.0 (0.90)	13.9 (0.99)
PEP:PHO	7.61 (2.10)	7.37 (3.30)	0.68 (0.15)	1.96 (0.44)	2.01 (0.35)
GLY:PEP	11.5 (2.89)	14.8 (8.74)	83.4 (48.0)	13.5 (1.87)	7.33 (0.77)
GLY:PHO	4.26 (0.38)	4.54 (0.79)	2.86 (0.18)	3.49 (0.24)	6.23 (0.66)

(6.48 nmol g^{-1} DW h^{-1}) (Table 5). SULF was correlated with the first canonical environmental gradient (Table 4, Fig. 3e).

The relative ratios of GLYC, PEPT, PHOS provide an estimate of nutrient limitations within the context of the UMRB reaches considered. The ratio of PEPT to PHOS in the five UMRB reaches was close to the expected 7:1 ratio for microbial N and P content (Chrzanowski and Keyle 1996; Hill et al. 2006; Cleveland and Liptzin 2007) in the two Mississippi River reaches. This ratio was much less than 7:1 in the remaining four UMRB reaches, suggesting some degree of P-limitation in these reaches (Table 5), though high within reach variability limits the strength of this conclusion. The ratios of GLYC to PEPT in the UMRB reaches, expected to be 8:1, suggest that only the upper Missouri River reach, with a ratio of 83:1, was C-limited, a point reinforced by the very low ratios of GLYC to PHOS, which were expected to be 60:1 (Table 5). Using conservative bounds (±50% of the 60:7:1 ratio), the extent of C, N, P-limitation in the five reaches of the UMRB were assessed. Overall, all five UMRB reaches experienced C-limitation based on enzyme activities, with 64–86% of the reaches exhibiting C-limitation. N-limitation was exhibited in only 1% of the UMRB reaches, but this proportion increased when C-limitation was ignored, resulting in N-limitation in 0–14% of all UMRB reaches. P-limitation was more prevalent (14–36% of all reaches), especially when C-limitation was factored out, and ranged from 86 to 100% of the UMRB reaches. No UMRB reach escaped some degree of nutrient limitation based on EEA (Table 3).

Discussion

Our estimates of EEA, and their relative activities among the various enzymes, are similar to values reported from the few other studies of microbial enzyme activity in large rivers (Sinsabaugh and Findlay 1995; Fischer et al. 2005); and from other aquatic ecosystems (Boschker and Cappenberg 1998; Ainsworth and Goulder 2000; Burns and Ryder 2001; Sinsabaugh and Foreman 2001; Harbott and Grace 2005). Most authors report enzyme activities dominated by phosphatase, leucine aminiopeptidase, and

β-glucosidase, as was generally the case for the rivers we studied.

The UMRB reaches in our study can be divided into three classes based on their relative EEA: (1) reaches in which phosphatase activity was greater than either peptidase or glycosidase activity; (2) reaches in which peptidases > phosphatases or glycosidases, and (3) reaches in which glycosidases > peptidases. River reaches exhibiting phosphatase dominance are presumed to be metabolically limited by relatively low P availability, as is the case for the Ohio River. This increase in phosphatase activity in response to declining or limiting P has been demonstrated in numerous aquatic environments (Cotner and Wetzel 1991; Kang and Freeman 1999; Nausch and Nausch 2000; Shackle et al. 2000; Wright and Reddy 2001). The second class is those river reaches dominated by peptidase activity (enzymes related to N acquisition and presumed to be metabolically constrained by relatively low N availability, as demonstrated by the Mississippi River reaches. As was the case with the phosphatases, several studies demonstrate increasing peptidase activity with declining N availability (Boschker and Cappenberg 1998; Montuelle and Volat 1998; Ainsworth and Goulder 2000; Nausch and Nausch 2000). The third class of UMRB reaches is those dominated by glycosidase activity (enzymes related to C acquisition) and presumed to be metabolically limited by relatively low C availability, as observed in the lower Missouri reach. Carbon supply is a well investigated constraint on microbial productivity and EEA (Sinsabaugh et al. 1997; Shackle et al. 2000; Burns and Ryder 2001; Sinsabaugh and Foreman 2001; Harbott and Grace 2005; Kang et al. 2005).

The activities of glycosidases, peptidases, and phosphatase explain only part microbial response to the nutrient environment of these rivers. A more comprehensive view of nutrient influences on microbial assemblages lies in the ratios, or stoichiometry of enzymes associated with C, N, and P acquisition. Ecological stoichiometric theory emphasizes importance of the balance of biologically important elements for regulating an organism's response to, and regulation of, their environment (Sterner and Elser 2002). The results of our comparisons of the stoichiometry of microbial enzyme activity with the molar ratios of C, N, and P in river waters and sediment suggest that microbial enzyme activity, while conceptually coupled with river chemistry, is influenced by factors other than surface water and sediment chemistry. Our chemistry measurements represent a single point in time and may not accurately reflect the C, N, and P history that has affected microbial enzyme activity, while our limited suite of enzyme assays do not account for physiological conditions of the microbial assemblage, including metabolic state and intra- and extracellular enzyme storage products, nor do they include all possible enzyme activities associated with nutrient acquisition. Both of these data gaps weaken the observed linkage between river chemistry and microbial enzyme activity (Wetzel 1991; Foreman et al. 1998; Sinsabaugh and Foreman 2003).

Three conclusions are evident from our comparison of approaches for estimating nutrient limitations based on the stoichiometry of these large floodplain rivers: (1) water chemistry and enzymes indicate that P-limitation is more prevalent than N-limitation; (2) the Ohio River reaches are more extensively P-limited than the Mississippi or Missouri River reaches; and (3) the N-limitation indicated by sediment chemistry is not reflected in the enzyme activities in the those same sediments, suggesting that the apparent N-limitation is most likely related to excess sediment C or P concentrations relative to sediment N concentrations (Table 3).

In addition to N and P-limitations, the great rivers or the UMRB exhibited extensive C-limitation based on water and sediment chemical stoichiometry and the ratio of glycosidase activity to either peptidase or phosphatase activity. Several studies in freshwater ecosystems report on the relationship between C-supply and EEA (Boschker and Cappenberg 1998; Foreman et al. 1998; Shackle et al. 2000; Harbott and Grace 2005). The general conclusion of these studies is that glycosidase and peptidase activity both increase with increasing organic matter content of sediments, a pattern seen in our data as well (Fig. 3). A corollary of this increasing sediment C content is that an increase in organic C complexity, being composed of a gradient ranging from a labile C pool to the more refractory cellulose and lignin C pools, dictates the relative activities of the glycosidases, peptidases, and phosphatases (Jackson et al. 1995; Sinsabaugh et al. 2002).

Sinsabaugh and Moorhead (1994) provided a model to better understand the ecosystem-level

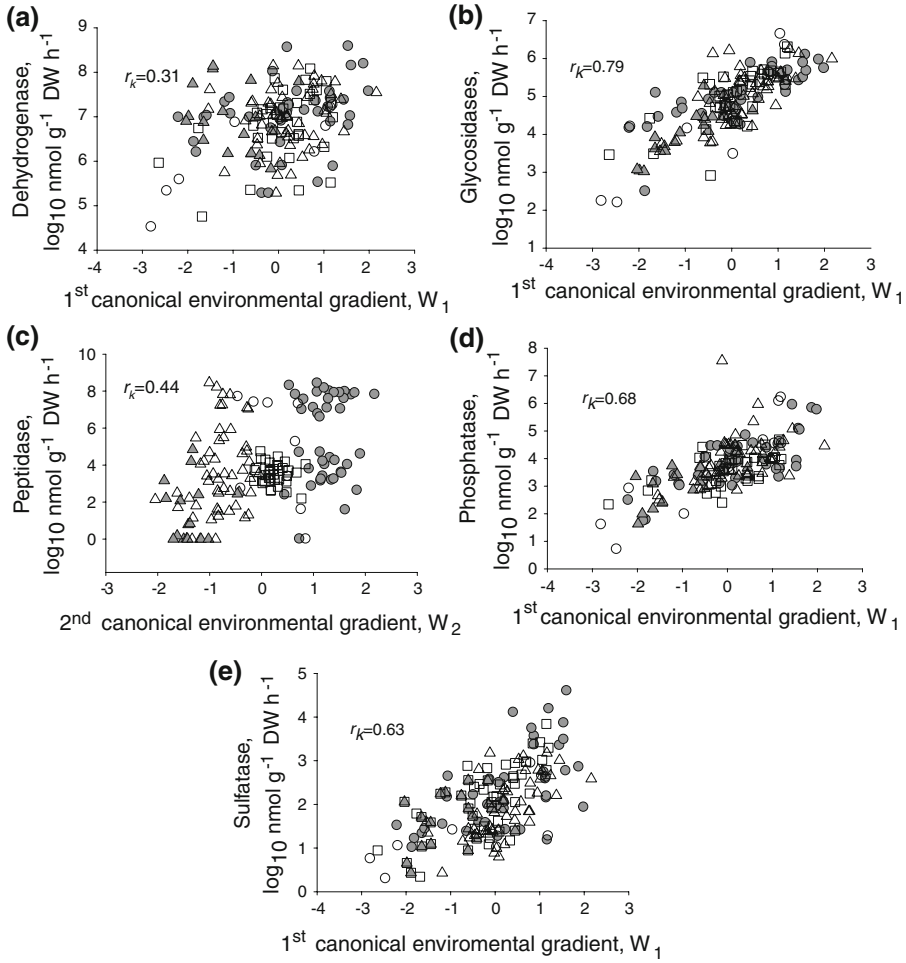

Fig. 3 The relationships between dehydrogenase (**a**), glycosidase (**b**), peptidase (**c**), phosphatase (**d**) and sulfatase (**e**) enzyme activities and the 1st and 2nd canonical environmental gradients for the impounded Mississippi (*shaded circles*), unimpounded Mississippi (*open circles*), upper Missouri (*shaded triangles*), lower Missouri (*open triangles*), and Ohio River (*open squares*) domains. Environmental variables included in the canonical gradient are presented in Table 4

interactions of organic matter and nutrients and how microbial processes mediate energy flow and nutrient cycling. Their model for microbial allocation of resources among community indicator enzymes (MARCIE) links organic matter decomposition with the activities of C-acquiring enzymes, the glycosidases. The glycosidases are constrained by the activities of N-acquiring (peptidases) and P-acquiring (phosphatases) enzymes. The relative activities of the functional classes of enzymes are therefore a measure of nutrient availability that may be used to assess such large-scale phenomena as regional impacts of climate change or anthropogenic disturbances (Sinsabaugh et al. 2002).

When viewed on a global scale, the Mississippi River is second only to the Amazon River in N export to the oceans, and third overall in P export (Howarth et al. 1996). What sets the Mississippi River apart from most of the world's major river basins is the percentage of its catchment in agriculture, especially corn and soybean row crops (Howarth et al. 1996; Goolsby et al. 1999; Donner et al. 2004). This is especially true for the UMRB where more than 30% of the basin is in agriculture, and where some of the major sub-basins exceed 50% of their catchments in agriculture. Two patterns that emerge from our data are the relatively higher N loads in the Mississippi River, especially the impounded reach, and the

relatively lower P loads in the upper Missouri and Ohio reaches. The land use influences of each basin are evident not only in the chemistries of each river, but also in the stoichiometry of microbial enzyme activities as the microbial assemblages of the rivers strive to balance relative N and P enrichments and organic carbon limitations to meet their physiological needs. The evidence from both chemistry and microbiology suggests that the impounded and unimpounded Mississippi reaches are relatively P-limited due to N-enrichment; the upper Missouri and Ohio reaches are relatively P-limited due to low P availability; and all river reaches are C-limited relative to N and P loads carried by the rivers. Allen and Gillooly (2009) suggest that one consequence of stoichiometric invariance, the preservation of elemental ratios among structural and metabolic constituents of organisms, may be that stoichiometric ratios of elements may be as important as absolute nutrient availability in constraining biological activity. As such, the stoichiometry of EEA may provide a biological perspective on the influence of anthropogenic land uses on river chemistry and the resulting imbalance of nutrients being transported from the UMRB catchments.

Our study linking microbial enzyme activities to regional-scale anthropogenic stressors in large river ecosystems suggests that microbial enzyme regulation of carbon and nutrient dynamics may be sensitive indicators of anthropogenic nutrient and carbon loading to these rivers. These findings are similar to those for wetlands (Hill et al. 2006) and for terrestrial soils (Sinsabaugh et al. 2008). The activities and relative ratios of microbial enzymes used in the acquisition of nutrients are inversely related to the overall supply and stoichiometric relations of the corresponding nutrients, especially in river sediments. Since nutrient concentrations in our river waters and sediments were correlated with the extent of agricultural land uses, EEA may also serve as indicators of these nutrient sources on a regional scale.

Acknowledgements We thank Xiaoli Yuan (USGS Upper Midwest Environmental Sciences Center) for analytical chemistry support; Marlys Cappaert and her data 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 design. 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

Ainsworth AM, Goulder R (2000) Downstream change in leucine aminopeptidase activity and leucine assimilation by epilithic microbiota along the River Swale, northern England. Sci Total Environ 251(252):191–204

Allen AP, Gillooly JF (2009) Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. Ecol Lett 12:369–384

American Public Health Association (APHA) (1998) Standard Methods for the Examination of Water and Wastewater, 20th ed. (eds. L.S. Clesceri, A.E. Greenberg & A.D. Eaton). American Public Health Association, Washington, DC

Angradi TR (ed) (2006) Environmental monitoring and assessment program, Great River ecosystems field operations manual. EPA/620R/06/002. US Environmental Protection Agency, Office of Research and Development, Washington, DC. http://www.epa.gov/emap/greatriver/fom.html. Accessed 1 July 2008

Angradi TR, Bolgrien DW, Jicha TM, Hill BH, Pearson MS, Taylor DL, Schweiger EW, Shepard L, Batterman AL, Batterman SL, Moffett MF, Elonen CE, Anderson LE (2009) A monitoring and assessment program for the Great Rivers of the Upper Mississippi River basin, USA. Environ Monit Assess 152:425–442

Baker JL (1985) Sources and fates of materials influencing water quality in the agricultural midwest—management practices to reduce farm chemical losses with agricultural drainage. In: Perspectives on nonpoint source pollution. EPA 440/5-85/001. US Environmental Protection Agency, Office of Water, Washington, DC, pp 467–470

Bedford BL, Walbridge MR, Aldous A (1999) Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80:2151–2169

Blenkinsopp SA, Lock MA (1990) The measurement of electron transport system activity in river biofilms. Water Res 24:441–445

Boschker HTS, Cappenberg TE (1998) Patterns of extracellular enzyme activities in littoral sediments of Lake Gooimeer, The Netherlands. FEMS Microbiol Lett 25:79–86

Broberg A (1985) A modified method for studies of electron transport system activity in freshwater sediments. Hydrobiologia 120:181–187

Burns A, Ryder DS (2001) Response of bacterial extracellular enzymes to inundation of floodplain sediments. Freshw Biol 46:1299–1307

Capone DG, Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: contrasts in anaerobic carbon catabolism. Limnol Oceanogr 33:725–749

Chrzanowski TH, Keyle M (1996) Ratios of carbon, nitrogen and phosphorus in *Pseudomonas fluorescens* as a model for bacterial element ratios and nutrient regeneration. Aquat Microb Ecol 10:115–122

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is the a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252

Cotner JB, Wetzel RG (1991) Bacterial phosphatases from different habitats in a small, hardwater lake. In: Chrost RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, New York, pp 60–83

Donner SD, Kucharik CJ, Foley JA (2004) Impact of changing land use practices on nitrate export by the Mississippi River. Global Biogeochem Cycles 18:1028–1048

Falkowski PG (2000) Rationalizing elemental ratios in unicellular algae. J Phycol 36:3–6

Fischer H, Kloep F, Wilzcek S, Pusch M (2005) A river's liver—microbial processes within the hyporheic zone of a large lowland river. Biogeochemistry 76:349–371

Foreman CM, Franchini P, Sinsabaugh RL (1998) The trophic dynamics of riverine bacterioplankton: relationships among substrate availability, ectoenzyme kinetics and growth. Limnol Oceanogr 43:1344–1352

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

Guy HP (1969) Laboratory analysis—laboratory theory and methods for sediment analysis. US Geological Survey Techniques of Water-Resources Investigations, Book 5, Chap C1, 58 pp

Harbott EL, Grace MR (2005) Extracellular enzyme response to bioavailability of dissolved organic C in streams of varying catchment urbanization. J North Am Benthol Soc 24:588–601

Hill BH, Herlihy AT, Kaufmann PR (2002) Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern USA. Freshw Biol 47:185–194

Hill BH, Elonen CE, Jicha TM, Cotter AM, Trebitz AS, Danz NP (2006) Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. Freshw Biol 51:1670–1683

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 Sens 70:829–840

Hoppe H (1991) Microbial extracellular enzyme activity: a new key parameter in aquatic ecology. In: Chrost RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, New York, pp 60–83

Howarth RW, Billen 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 nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry 35:75–139

Hutchinson GE (1971) A treatise on limnology. Volume. I. Geography, physics, and chemistry. Wiley, New York

Jackson CR, Foreman CM, Sinsabaugh RL (1995) Microbial enzyme activities as indicators of organic matter processing in a Lake Erie coastal wetland. Freshw Biol 34: 329–342

Kang H, Freeman C (1999) Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. Soil Biol Biochem 31:449–454

Kang H, Freeman C, Park SS, Chun J (2005) *N*-acetylglucosaminidase activities in wetlands: a global survey. Hydrobiologia 532:103–110

Montuelle B, Volat B (1998) Impact of wastewater treatment plant discharge on enzyme activity in freshwater sediments. Ecotoxicol Environ Saf 40:154–159

National Atmospheric Deposition Program (2008) National atmospheric deposition program. Illinois State Water Survey, Champaign, IL. http://nadp.sws.uiuc.edu/

National Research Council (2002) The Missouri River ecosystem: exploring the prospects for recovery. Committee on Missouri River Ecosystem Science. National Research Council, Washington, DC, p 173

Nausch M, Nausch G (2000) Stimulation of peptidase activity in nutrient gradients in the Baltic Sea. Soil Biol Biochem 32:1973–1983

Packard TT (1971) The measurement of respiratory electron transport activity in marine phytoplankton. J Mar Res 29:235–244

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221

Rohlf FJ, Sokal RR (1969) Statistical tables. WH Freeman and Company, San Francisco

Shackle VJ, Freeman C, Reynolds B (2000) Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biol Biochem 32:1935–1940

Sinsabaugh RL, Findlay SG (1995) Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb Ecol 30: 127–141

Sinsabaugh RL, Foreman CM (2001) Activity profiles of bacterioplankton in a eutrophic river. Freshw Biol 46: 1239–1249

Sinsabaugh RL, Foreman CM (2003) Integrating dissolved organic matter metabolism and microbial diversity: an overview of conceptual models. In: Findlay SG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, New York, pp 425–454

Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biol Biochem 26:1305–1311

Sinsabaugh RL, Findlay S, Franchini P, Fisher D (1997) Enzymatic analysis of riverine bacterioplankton production. Limnol Oceanogr 42:29–38

Sinsabaugh RL, Carreiro MM, Alvarez S (2002) Enzyme and microbial dynamics of litter decomposition. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity ecology & applications. Marcel Dekker, Inc, New York, pp 249–265

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ

Sundareshwar PV, Morris JT, Koepfler E, Fornwalt B (2003) Phosphorus limitation of coastal ecosystem processes. Science 299:563–565

US Geological Survey (2001) National land-cover database for the United States (2001) US Geological Survey, Reston, Virginia. http:\\www.mrlc.gov

Trevors JT, Mayfield CI, Inniss WE (1982) Measurement of electron transport system (ETS) activity in soil. Microb Ecol 8:163–168

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, Dortch Q (2003) Future aquatic nutrient limitations. Mar Pollut Bull 46:1032–1034

Wetzel RG (1991) Extracellular enzymatic interactions: storage, redistribution, and interspecific communication. In: Chrost RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, New York, pp 6–28

Wright AL, Reddy KR (2001) Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci Soc Am J 65:588–595