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Algal biomass accrual in relation to nutrient availability and limitation along a longitudinal gradient of a karst riverine system

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Abstract

Nutrient availability influences growth, productivity, and community structure of primary producers. Nutrient limitation, however, results from a deficiency mainly in nitrogen (N) or phosphorus (P) levels relative to cellular growth needs. Limitation is a function of biotic and abiotic factors, the latter including land-use activities (e.g., agriculture, septic systems) and underlying bedrock features. The purpose of this study was twofold: (1) to assess the relationship between algal biomass and ambient nutrient levels along the longitudinal course of a river through a transition from weak to well-developed underlying karst bedrock and (2) experimentally assess if periphyton was N- or P-limited between weak and well-developed karst reaches. Sestonic and *Cladophora* biomass (=chlorophyll-*a*) levels increased sharply along the longitudinal gradient. *Cladophora* biomass, in particular, was strongly correlated with nitrate levels. In contrast, periphyton biomass (=chlorophyll-*a*) levels were sporadic and did not display a longitudinal pattern. With the exception of ammonia, individual nutrient levels generally increased longitudinally and were higher in the downstream karst reaches. Total N/total P ratios also increased longitudinally and were >25 throughout the study region, suggesting P limitation. The results of the nutrient limitation studies, however, coupled with high concentrations of both N and P throughout the study reach in excess of eutrophication thresholds, suggest that total nutrients are not limiting within the study region. Overall, Kentucky's upper Green River appears to be a nutrient-enriched, eutrophic system and particularly in the downstream, well-developed karst reaches.

Keywords: Algae, Nutrients, Phosphorous, Nitrogen, Nitrate, Karst, *Cladophora*

Background

Nutrient limitation operates across a broad variety of ecosystems, occurring when a nutrient is in short supply relative to cellular growth requirements (Dodds et al. 2002). Primary productivity and algal biomass accrual are typically higher in response to greater nutrient availability (Lohman et al. 1991). Nutrients also influence algal community structure, and individual species often have different nutrient requirements (Hecky and Kilham 1988; Borchardt 1996). Baysinger-Daniel (1989) found that filamentous species were more common in nutrient-enriched sites. In contrast, low-nutrient sites are typically devoid of filamentous, macroalgal taxa and dominated mainly by diatom and cyanobacteria assemblages (Lohman et al. 1991).

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The most common limiting nutrients in aquatic ecosystems are nitrogen (N) and phosphorus (P) (Elser et al. 2001; Dodds 2003). In a literature survey of 200 temperate streams from North America and New Zealand, Dodds et al. (2002) found that N limitation, P limitation, and N and P colimitation occur. Dodds and Welch (2000) compiled the data from 158 limitation bioassays, of which 13% were stimulated by N, 18% by P, 44% by both N and P, and 25% by neither nutrient. Rosemond et al. (1993) found that the limiting nutrient varied annually, and small changes in the concentration of one limiting nutrient induced limitation by the other. Both N and P limitations can occur due to alterations of algal community structure (Tank and Dodds 2003).

Weathering of underlying geology was once considered a minor contributor to nutrient availability (Grimm et al. 2003). Lohman et al. (1991) investigated stream nutrient levels across the USA and found that P limitation was common east of the Mississippi River and N limitation prevalent in the Pacific Northwest. Grimm et al. (2003) asserted that the assumption of P limitation in freshwater was geographically biased since most prior research had been conducted in the eastern USA in areas with low-P parent geology and excessive N inputs from agriculture. Holloway et al. (1998) reported that exposed sedimentary rocks contain 20% of the global N inventory, and when the stream bedrock has high nutrient (e.g., NO_3) levels, the underlying geology becomes a major contributor to the stream channel. Dodds and Welch (2000) similarly found that phosphate-rich rocks may provide sufficient P to prevent limitation. Groundwater may also contribute to in-stream nutrient levels. In karst geologic regions, nutrient-enriched groundwater can mix with the surface water via springs and fractures in the bedrock (Notestein et al. 2003).

The purpose of this study was twofold: (1) to assess the relationship between ambient algal biomass and in-stream nutrient levels along the longitudinal transition of a river from weak to well-developed underlying karst bedrock and (2) experimentally assess if periphyton was N- or P-limited between weak and well-developed karst reaches.

Methods

Study site descriptions

This research was conducted during summer baseflow conditions in six reaches along the upper Green River between the Green River Lake (GRL) and Mammoth Cave National Park, KY, USA (Figure 1). The Green River flows west to its confluence with the Ohio River, passing through Level III Ecoregions 71 (Interior Plateau) and 72 (Interior River Valley and Hills) (Woods et al. 2002). The four upstream reaches (1 to 4) were positioned in the Eastern Highland Rim Level IV Ecoregion (71 g), characterized by shale, sandstone, and weakly developed karst topography. The two downstream reaches were located in the Western Pennyroyal Karst Plain (71 e, reach 5) and Crawford-Mammoth Cave Uplands (71 a, reach 6) Level IV Ecoregions. Both are underlain by Mississippian limestone and fractured bedrock with very low surface stream density and N-rich groundwater.

Water from deep-release reservoirs often contain high nutrient concentrations (Marcus 1980), directing us to establish our most upstream reach far enough downstream from GRL to minimize an impoundment influence. Prior sampling revealed that ammonia and sestonic chlorophyll-*a* levels were much higher within the first 20 km downstream of GRL. Reach 1 is 24 km downstream from the GRL outlet (Table 1). All six reaches were characterized by an open canopy and shallow run habitats underlain by cobble and gravel substrates.

Figure 1 Map depicting the location of the upper Green River and Green River Lake, KY, USA.
Sampling reaches are marked by numbers and solid black circles.
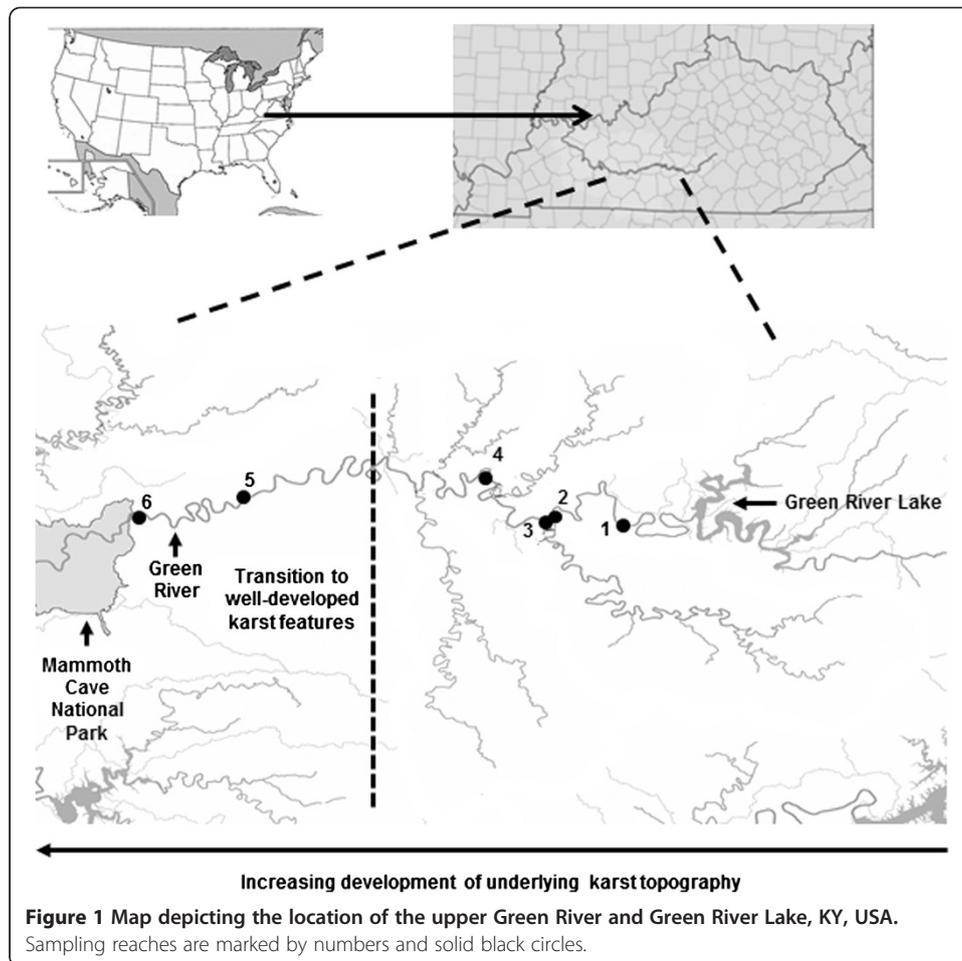
Field and laboratory methods

Longitudinal trends: environmental parameters

Environmental parameters were quantified in July to August 2008 and July to August 2009. Current velocity was measured with a Marsh-McBirney Flo-Mate velocity meter (Frederick, MD, USA) at each reach during each sampling event, and discharge was quantified using the velocity-area method (Gore 2007). HOBO® Water Temp Pro v2 Logger temperature probes (Bourne, MA, USA) were used to measure the temperature at 1-h intervals. Grab

Table 1 Summary of in-stream physical parameters in six study reaches along the upper Green River, KY, USA

	Reach					
	1	2	3	4	5	6
Distance from GRL (km)	24	43	47	64	130	155
Mean wetted width (m)	34.9	26.8	29.9	34.5	47.5	47.8
Mean depth (cm)	60	54	67	69	86	93
Mean discharge (m³/s)	1.70	1.90	1.70	1.80	5.00	5.80
Mean temperature (°C)	27.1	26.4	27.5	27.3	24.1	24.9
Temperature range (°C)	22.3 to 31.3	21.9 to 30.8	22.2 to 31.7	21.4 to 31.5	19.3 to 27.6	18.8 to 28.1

GRL, Green River Lake.

samples for the measurement of ambient nutrient levels were obtained at midstream in acid-washed 275-mL bottles. Three (2008) or four (2009) replicate samples were analyzed per site. Total phosphorus (TP; acid persulfate digestion), soluble reactive phosphorus (SRP; ascorbic acid method), nitrate (cadmium reduction method), ammonia (salicylate method), and total nitrogen (TN; persulfate digestion method) levels were quantified spectrophotometrically (APHA 1998).

Longitudinal trends: algal biomass

Periphyton and sestonic algal biomass were quantified in 2008; periphyton, sestonic, and *Cladophora* biomass, in 2009. Ten replicate samples were taken for each algal type. Grab samples to quantify sestonic algal levels were obtained at midstream in acid-washed 275-mL bottles. Periphyton and *Cladophora* samples were taken from two randomly selected quadrats positioned along five separate cross-stream transects (n = 10 total). Periphyton samples were obtained from cobble substrates found in the downstream left quarter of each quadrat. A penny was used to establish a 3.8-cm^2 circle at the rock center; periphyton within the circle was scraped with a scalpel and toothbrush (Steinman and Lamberti 1996), rinsed with stream water into a small pan, and transferred into acid-washed 275-mL bottles (Dodds 2003). A new transect was established 1 to 2 m upstream, and new quadrats were selected for *Cladophora*. Algae were clipped from a 75-cm^2 area subquadrat located in the upstream right corner of the quadrat, placed in a plastic bag, and put on ice in a cooler. All algal samples were refrigerated up to 24 h prior to processing.

Periphyton and sestonic algal samples were vacuum-filtered on 47-mm-diameter, 0.7-μm pore size Whatman GF glass-fiber filters, placed in individual Petri dishes, wrapped in foil, and frozen at –10°C in the dark for up to 14 days. *Cladophora* samples were prepared as a slurry by mixing in a set volume (e.g., 500 mL) of di-H_2O in a heavy-duty blender and filtered as above. Algal samples were analyzed for chlorophyll-*a* concentration using USGS methods (Yin 2005). Each filtered sample was placed in a 50-mL centrifuge tube with 3 to 6 glass beads and 10 mL of 50:50 dimethyl sulfoxide (DMSO)-acetone, vortexed for 30 s, stored overnight in the dark at 4°C, centrifuged 10 min at 4,300 rpm, and the supernatant was transferred to a new tube. A second 10 mL of DMSO-acetone was added to the original sample, and the process was repeated. The two supernatant liquids were combined. A final 5 mL of DMSO-acetone was added to the original sample, and the process was repeated. The resulting combined supernatant was centrifuged, and 5 mL of the liquid was analyzed with a Shimadzu RF-5301 PC spectrofluorometer (Nakagyo-ku, Kyoto, Japan). Samples were measured against set chlorophyll-*a* standards produced from an initial 239 ppb stock solution and 20% serial dilutions of 47.60, 9.52, 1.90, and 0.38 ppb. A linear regression of intensity vs. concentration of the standards established a standard curve used to calculate chlorophyll concentrations for sestonic (mg/L) and periphyton and *Cladophora* biomass (mg/m^2) samples.

Nutrient limitation study: weak vs. well-developed karst systems

Nutrient-diffusing substrates (NDS; Carey et al. 2007) were deployed in August to September 2009 at two reaches (1 and 6) with contrasting karst geology and nutrient conditions (e.g., Figure 2D). Forty LDPE vials were filled with ten replicates each of four nutrient solutions (control, N = 87.5 mg/L NO_3^- using 632 mg/L KNO_3, P = 12 mg/L PO_4^- using 103.8 mg/L Na_2HPO_4, and N + P) and attached to a platform in a randomized pattern. Nutrients diffused through a glass fiber filter (Whatman, Clifton, NJ, USA; 24-mm diameter, 0.7-μm pore size) and a membrane filter (Millipore Co., Billerica,

Figure 2 Summer baseflow nutrient and TN/TP levels. Summer baseflow nutrient (mg/L) and TN/TP levels in the upper Green River with increasing distance from the Green River Lake (km). (**A**) Total phosphorus, (**B**) soluble reactive phosphorus, (**C**) total nitrogen, (**D**) nitrate, (**E**) ammonia, and (**F**) TN/TP.
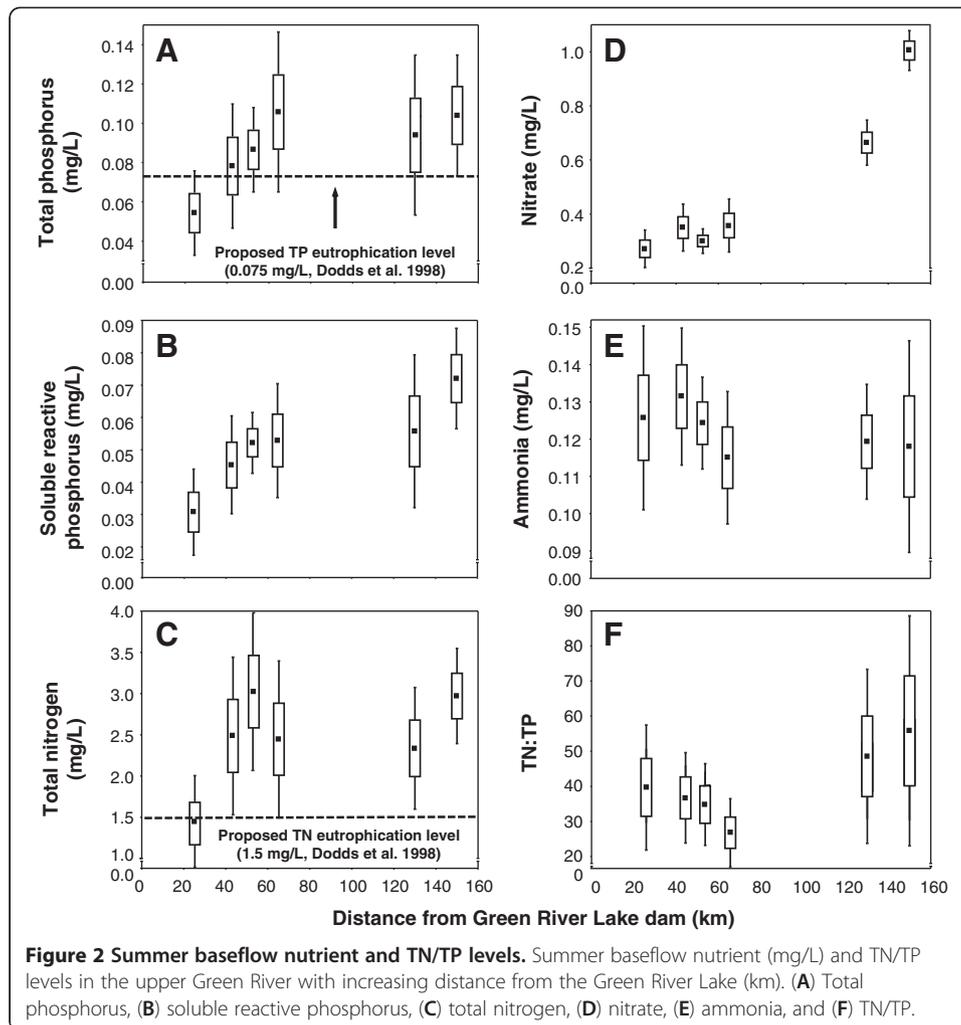
MA, USA; 25-mm diameter, 0.45-μm pore size). Fiber filters served as periphyton growth surfaces. The NDS were anchored to the streambed and retrieved after 15 days. Fiber filters were frozen at −10°C in the dark for up to 14 days and analyzed for chlorophyll-*a* biomass (mg/m^2) as described above (Yin 2005).

Statistical methods

Pearson product–moment correlation analyses were used to assess the relationship between chlorophyll-*a* biomass of each algal type and ambient nutrient levels. A two-way factorial analysis of variance (ANOVA) compared NDS periphyton chlorophyll-*a* biomass between the control and the three nutrient addition (N, P, and N + P) treatments to assess limitation. The critical α-level was reduced to 0.05/28 (=0.00179) due to the total number of pair-wise comparisons. Pearson correlations and the ANOVA were performed using Statistica 7.0 (StatSoft®, Tulsa, OK, USA).

We employed a simple arithmetic technique to evaluate the relative magnitude of the response by periphyton with the nutrient limitation study. We calculate a treatment biomass response relative to the control (Brett and Goldman 1997; Tank and Dodds

2003). This was performed as the log ratio of each nutrient addition treatment compared to the control (N/C, P/C, and N + P/C). A ratio equal to 0 implies that ambient nutrient levels are sufficient to support periphyton growth. Ratios were calculated separately for the upstream and downstream reaches.

Results

Longitudinal trends: environmental parameters and algal biomass

Stream size, discharge, and thermal conditions of the four upstream reaches were generally similar (Table 1). Thermal conditions, in particular, of these reaches were *ca.* 2°C to 3°C warmer than the two downstream karst reaches. Nutrient and algal data were pooled and plotted across the four sampling periods to smooth out variability due to small between-month hydrological changes. Except for ammonia, there was a general trend of increasing nutrient content with increasing distance from the GRL dam (Figure 2). TP, SRP, TN, and nitrate levels were markedly higher in the downstream karst reaches (Figure 2A,B,C,D). Nitrate levels, in particular, in the most downstream reach were *ca.* three- to fourfold higher compared to the upstream reaches (Figure 2D). Levels of TN and TP were nearly twofold higher in the downstream karst reaches. Consequently, TN/TP ratios were also highest in the downstream karst reaches (Figure 2F).

Sestonic algal biomass levels were lowest in the three upstream reaches, *ca.* three- to fourfold lower compared to the downstream karst reaches (Figure 3A). There was no clear longitudinal trend with periphyton biomass (Figure 3B). *Cladophora* was mostly absent from the upstream reaches but present only in scattered patches of short strands that were not in the sampling transects (Figure 3C). In contrast, by late summer, *Cladophora* was present as dense, cross-channel mats in the downstream karst reaches. Sestonic algal biomass was linearly related to both SRP and TP levels, but not any of the N parameters (Table 2). *Cladophora* biomass was strongly correlated only with nitrate levels, but no linear relationships were evident between periphyton biomass and any nutrient (Table 2).

Nutrient limitation: weak vs. well-developed karst reaches

Mean chlorophyll-*a* values from the NDS treatments were generally higher at the upstream, weakly developed karst reach (Figure 4 and Table 3). Although there were two significant, pair-wise differences, there were no within-reach differences. Periphyton biomass on the upstream N + P (0.46-mg/L chlorophyll-*a*) treatment was significantly higher than both the downstream control (0.29 mg/L, $p = 0.0007$) and downstream N- (0.30 mg/L, $p = 0.0011$) addition treatments (Table 3). Growth on the upstream N + P vials also slightly exceeded growth on other upstream treatment vials, but not to a significant degree. The same was evident for the downstream N + P treatment compared to the downstream control, *N*, and P treatments.

Biomass response ratios for N (N/C) and P (P/C) at the upstream reach were both negative (Table 4), indicating that neither single nutrient-addition treatment enhanced periphyton growth. The dual-nutrient N + P/C ratio (=0.05) similarly suggested only a very minor periphyton response. The downstream N/C ratio was marginally greater than 0, and this also suggests ample ambient N availability (Table 4). The downstream P/C ratio was somewhat higher (0.13), suggesting there was only a minor periphytic response to P enhancement. The dual N + P/C ratio was nearly identical to the P/C ratio.

Figure 3 Summer baseflow sestonic algal (A, µg/L), periphyton (B, mg/m²), and *Cladophora* (C, mg/m²) biomass levels. In the upper Green River with increasing distance from the Green River Lake (km).
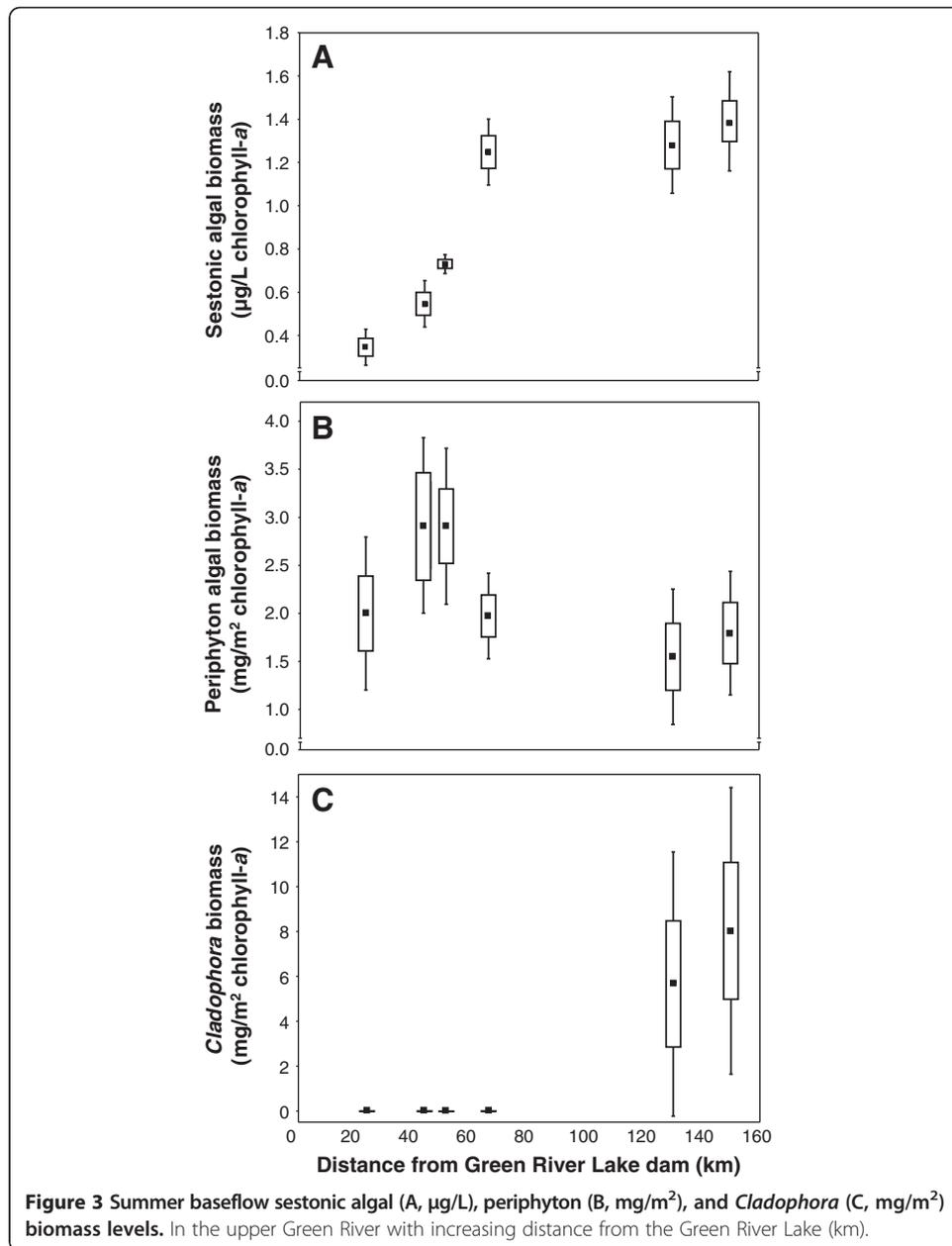
Table 2 Summary of Pearson correlation analyses between algal biomass levels and ambient nutrient levels

Nutrient	Algal type		
	Sestonic	Periphyton	*Cladophora*
TP	$r = 0.91$, $p = 0.011$	ns	ns
SRP	$r = 0.83$, $p = 0.042$	ns	ns
TN	ns	ns	ns
NIT	ns	ns	$r = 0.98$, $p = 0.004$
AMM	ns	ns	ns
TN/TP	ns	ns	ns

TP, total phosphorus; SRP, soluble reactive phosphorus; TN, total nitrogen; NIT, nitrate; AMM, ammonia; ns, nonsignificant ($p > 0.05$).

Figure 4 Chlorophyll-*a* biomass accrual (mg/m²) on the glass fiber filters in the NDS treatments.
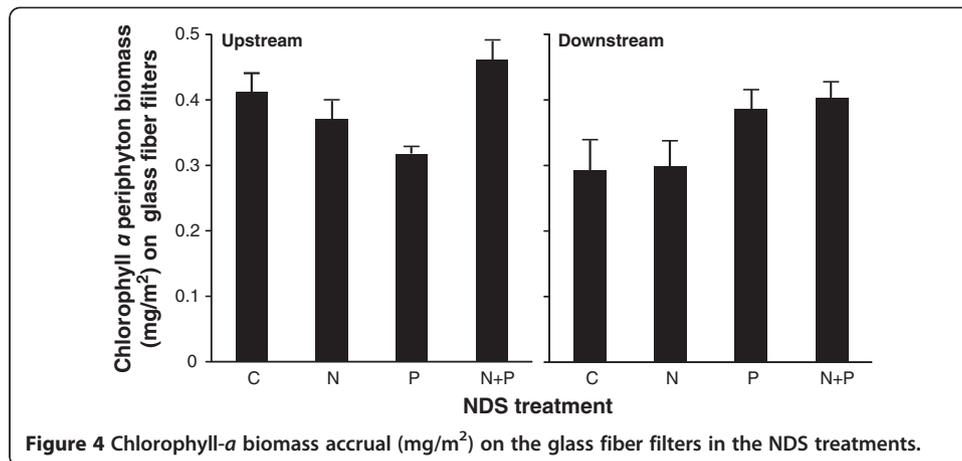
Discussion

Longitudinal patterns: nutrients and algae

Spatial patterns of in-stream nutrient levels can result from abiotic (e.g., surface and groundwater inputs, land-use patterns) and biotic (e.g. microbial uptake) factors. Nitrogen levels in aquatic systems are usually low, yet higher in watersheds where land is mainly used for agriculture (Zhang et al. 1996; Frankforter et al. 2009). Nitrate enrichment of groundwater can occur due to nonpoint inputs from commercial and residential fertilizers (Böhlke and Denver 1995; Notestein et al. 2003). Panno et al. (2001) found that nitrate contamination in karst aquifers was predominantly from N fertilizers and much less from atmospheric deposition or human and animal wastes. Almasri and Kaluarachchi (2004) showed that high nitrate levels in groundwater occurred in areas with high recharge rates and high surface nitrogen loadings. We contend that the high ambient nutrient levels in the downstream karst reaches of the upper Green River, particularly nitrate, are reflective of a combination of agricultural land-use activities and underlying bedrock conditions with concomitant karst groundwater inputs. Land adjacent to the upper Green River is partially in agriculture uses, namely row cropping and small livestock operations (Grubbs et al. 2007).

Table 3 Pair-wise results of the two-way factorial ANOVA

Treatment	Pair-wise probabilities						
	U/N	U/P	U/N + P	D/C	D/N	D/P	D/N + P
U/C	1	1	1	0.0136	0.0196	1	1
U/N		1	1	1	1	1	1
U/P			0.0032	1	1	1	1
U/N + P				0.0007*	0.0011*	1	1
D/C					1	1	0.0225
D/N						1	0.0321
D/P							1
D/N + P							

Values are pair-wise probabilities comparing NDS chlorophyll-*a* periphyton biomass levels (mg/m²) between the control and nutrient-addition treatments. U, upstream; D, downstream; C, control; N, nitrogen; P, phosphorus. *Pair-wise significant difference at $p < 0.00179$.

Table 4 Summary of biomass response ratio values

Ratio	Upstream	Downstream
N/C	−0.04	0.01
P/C	−0.11	0.13
N + P/C	0.05	0.14

Response ratio values compare mean chlorophyll-*a* biomass (mg/m^2) of each nutrient-addition treatment relative to the control.

Highly permeable karst geology provides multiple, fractured conduits for groundwater to rapidly mix with surface waters (Katz et al. 1997). Weathering of soils and bedrock can be natural sources of nutrients (Holloway et al. 1998; Morford et al. 2011). Surface runoff from topsoil and organic matter, plus dissolution of bedrock by groundwater flow, are natural sources of N to surface water and groundwater. In the karst region, surface runoff enters the dense network of subsurface conduits where there is relatively little filtration, biological uptake, or degradation of nutrients or other nonpoint source pollutants (Ryan and Meiman 1996). Springs can also discharge nutrient-rich water directly in the upper Green River. Spring systems in the karst region of the upper Green River typically exhibit high nitrate levels (U.S. Geological Survey 2001), with several individual systems exceeding 4 mg/L (data not shown). Hill (1981) noted that saltpeter (KNO_3) deposits in the Mammoth Cave originated from nitrates leached from limestone bedrock. Holloway et al. (1998) found that a California watershed with high bedrock concentrations of N contributed up to 90% of the nitrates in downstream reservoirs. A geochemical by-product of enhanced nitrate enrichment in lotic systems, however, is the inefficiency to denitrify excess loading (Mulholland et al. 2008), leading to downstream nutrient accumulation and potentially harmful algal blooms and hypoxic conditions (Ohte 2012).

Except for ammonia, nutrient levels in this study increased longitudinally along the upper Green River with corresponding downstream increases in sestonic algal and *Cladophora* chlorophyll-*a* levels. With the exception of the most upstream reach in the weak karst region, both TN (>1.5 mg/L) and TP (>0.075 mg/L) levels easily exceeded the mesotrophic-eutrophic boundary threshold proposed by Dodds et al. (1998). Individual TN levels in this study ranged from 0.5 to 4.3 mg/L, markedly higher than the benthic algal biomass accrual breakpoint of 40 µg/L TN illustrated by Dodds et al. (2002). The combination of the high nutrient levels and rapid accrual of thick *Cladophora* mats during late summer in the karst reaches are strongly indicative of eutrophic conditions. In contrast, mean sestonic chlorophyll-*a* levels in the karst reaches were only 1.3 to 1.4 µg/L (=mg/m^3) and markedly less than the 8- to 10-mg/m^3 eutrophic threshold suggested by Dodds (2006).

The sharp differences in *Cladophora* biomass between the upstream and downstream reaches may be a function of a combination of biological, physical, and chemical factors. There have been several studies that have shown that *Cladophora* fragments/filaments have been present in the gut tracts of aquatic macroinvertebrate taxa (e.g., Rhame and Stewart 1976; Furey et al. 2012). Tinsley (2012) recently demonstrated using $\delta^{13}C$ stable isotope data that *Cladophora* was the prominent assimilated dietary item during summer for two hydropsychid caddisflies. What appears lacking from the upper Green River, however, are grazer communities that markedly reduce standing stocks of *Cladophora*. The upper Green River supports dense communities of gastropod snails, mainly *Leptoxis praerosa* (Say), that inhabit *Cladophora* beds during summer, yet individuals are typically

in higher numbers on coarse woody debris and cobble-sized substrates (Grubbs, unpublished data). In addition, $\delta^{13}C$ stable isotope data for *L. praerosa* during baseflow conditions with high *Cladophora* standing stocks showed that the snails were assimilating a heterogeneous mix of the filamentous alga and both algal and detrital components of epilithic biofilm (Grubbs, unpublished data). Other potential grazers (e.g., glossosomatid and *Helicopsyche* caddisflies) that have been shown to graze on *Cladophora* in other systems (Feminella and Resh 1991; Sturt et al. 2011) are either lacking or in very low numbers in the upper Green River.

Thick accumulations of macroalgae, especially *Cladophora*, are typically associated with ample light availability, stable hydraulic conditions, and high nutrient levels in a broad variety of aquatic ecosystems (Dodds and Gudder 1992). *Cladophora* can also positively respond to experimental nutrient additions (Lapointe and O'Connell 1989). All the reaches in this study have an open canopy with ample light, suggesting that the lack of *Cladophora* in the upstream reaches was not due to light limitation. During baseflow conditions, rapid growth of lotic *Cladophora* in nutrient-enriched systems can lead to large floating, cross-channel mats (Busse et al. 2006; Power et al. 2009). In this study, the rapid accrual of *Cladophora* biomass in the downstream reaches occurred in July to August 2009 when the Green River approached baseflow conditions. *Cladophora*, however, can also be easily removed from coarse substrates during periods of hydrologic instability. Mass sloughing can occur in tide pools due to wave activity (Dethier 1982) and in riverine systems because of amplified flow rates (Entwisle 1989; Busse et al. 2006). Much of the *Cladophora* growth in this study was removed by late October.

Cladophora can also be heavily colonized by microscopic, epiphytic diatoms (Peterson and Grimm 1992) that harbor N-fixing cyanobacteria that take up dissolved N and may provide a supplementary NH_3 source (Power et al. 2009) to the water column. With the large potential habitat for N-fixing cyanobacteria provided by *Cladophora*, it is easy to speculate high N-fixation rates and accumulation of NH_3 in the downstream, karst reaches. While nitrate levels increased longitudinally, the levels of NH_3 in the upper Green River are relatively stable.

Nutrient limitation of weak and well-developed karst systems

Nitrogen and phosphorous availability may limit primary productivity, with co-limitation occurring commonly (Corkum 1996; Tank and Dodds 2003; Harpole et al. 2011). Periphyton chlorophyll-*a* biomass levels on the NDS growth filters were significantly higher with the upstream N + P treatment, but only compared to the downstream control and N-addition treatment. The lack of within-reach significance between the control and any nutrient-addition treatment implies a lack of nutrient limitation for both reaches. The negative N/C and P/C ratio values in the upstream reach, plus the very small N + P/C value, also indicates that periphyton responses were stimulated to a minor degree only by the dual nutrient treatment. Although the upstream reach had the lowest ambient N and P levels and was also the only not classified as a eutrophic system according to the criteria established by Dodds et al. (1998), ample nutrient content may still have been present for the short-term establishment of periphyton communities.

Molar TN/TP ratios >20 imply P limitation (Grimm and Fisher 1986; Busse et al. 2006). Using this criterion alone, all six upper Green River reaches are P limited since the lowest mean TN/TP ratio value was 24.9 (reach 4) and nearly 40 in the most

upstream reach. The lack of nutrient limitation revealed with both NDS studies, however, suggests that ambient nutrient levels exceeded a threshold to induce a significant periphyton response. Not surprisingly, in the nutrient-enriched far downstream reach, periphyton responded only minimally to the N and P additions in the NDS treatments. Dodds et al. (2002) have suggested that nutrients may no longer limit algal growth at very high nutrient levels regardless of N/P ratios.

Conclusions

Kentucky's upper Green River is a nutrient-enriched system that we attribute mainly to a combination of agricultural land-use and limestone bedrock characteristics that lead to enriched groundwater inputs in the downstream, well-developed karst reaches. Measurements of nitrate, TN, TP, and algal data, particularly the dense *Cladophora* mats that proliferated during summer, revealed that the study system is eutrophic with the possible exception of the most upstream reach. The lack of periphyton biomass responses to the NDS treatments, especially in light of molar TN/TP ratios >20, suggested that the study system is nutrient saturated and not nutrient limited.

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