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PRIMARY RESEARCH PAPER

Nutrient limitation of biofilm biomass and metabolism in the Upper Snake River basin, southeast Idaho, USA

Amy M. Marcarelli · Heather A. Bechtold · Amanda T. Rugenski · Richard S. Inouye

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Abstract It is essential to know the nutrient limitation status of biofilms to understand how they may buffer uptake and export of nutrients from polluted watersheds. We tested the effects of nutrient additions on biofilm biomass (chlorophyll *a*, ash free dry mass (AFDM), and autotrophic index (AI, AFDM/chl *a*)) and metabolism via nutrient-diffusing substrate bioassays (control, nitrogen (N), phosphorus (P), and N + P treatments) at 11 sites in the Upper Snake River basin (southeast Idaho, USA) that differed in the magnitude and extent of human-caused impacts. Water temperature, turbidity, and dissolved inorganic N concentrations all changed seasonally at the study sites, while turbidity and dissolved inorganic N and P also varied with impact level. Chl *a* and AI on control treatments suggested that the most heavily impacted sites supported more autotrophic biofilms than less-impacted sites, and that across all sites biofilms were more heterotrophic in autumn than in summer. Nutrient stimulation or suppression of biofilm biomass was observed for chl *a* in 59% of the experiments and for AFDM in 33%, and the most frequent response noted across all study sites was N limitation. P suppression of chl *a* was observed only at the most-impacted sites, while AFDM was never suppressed by nutrients. When nutrient additions did have significant effects on metabolism, they were driven by differences in biomass rather than by changes in metabolic rates. Our study demonstrated that biofilms in southeast Idaho rivers were primarily limited by N, but nutrient limitation was more frequent at sites with good water quality than at those with poor water quality. Additionally, heterotrophic and autotrophic biofilm components may respond differently to nutrient enrichment, and nutrient limitation of biofilm biomass should not be considered a surrogate for metabolism in these rivers.

Keywords Eutrophic river · Nutrient limitation · Biofilm metabolism · Nutrient loading · Snake River

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Introduction

Anthropogenic impacts can profoundly influence the structure and function of streams and rivers. One of the most widespread effects of human activities is an increase in nutrient concentrations via both point and non-point source loading (e.g., Royer et al., 2006). Increased nutrient loads may have direct effects on the nutrient uptake capacity of river ecosystems by altering their structural and functional attributes (Young & Huryn, 1999; Paul & Meyer, 2001; Meyer et al., 2005). Meyer et al. (2005) argued that the increased nutrient concentrations and export commonly associated with urban streams result from the complex interactions between increasing nutrient loads and decreased biological uptake capacity. There is a need to understand how biological processes interact with and affect nutrient concentrations in watersheds, as nutrients that are exported from one watershed are imported into reservoirs, rivers, and estuaries downstream (Royer et al., 2006).

Nitrogen (N) and phosphorus (P) commonly limit biofilm biomass in streams (Francoeur, 2001; Tank & Dodds, 2003; Elser et al., 2007). Knowledge of how nutrients limit biofilm biomass and metabolism is key to understanding the effects of increased nutrient loads on stream ecosystems, and can help direct eutrophication management by focusing activities on reducing concentrations of the limiting nutrient (e.g., Schindler, 1974; Dodds & Welch, 2000). This approach has been successfully used to manage lake eutrophication (Edmondson & Lehman, 1981). If biofilm communities are nutrient limited, they can increase growth and/or production in response to additional nutrient loads, removing nutrients from the water column and storing them (albeit temporarily) as biomass. Nutrient saturation occurs when nutrient loads exceed the biotic uptake capacity of ecosystems, leading to increased nutrient export from watersheds (Stoddard, 1994; Bernot & Dodds, 2005; Earl et al., 2006). In streams where heterotrophs (fungi and bacteria) are important members of biofilms, nutrient additions have been observed to suppress algal biomass (Bernhardt & Likens, 2004), and heterotrophs and autotrophs may be limited by different nutrients in the same stream (Tank & Dodds, 2003).

Despite the interest in developing nutrient criteria to manage eutrophication in streams (Dodds & Welch, 2000), there have been few direct tests of the effects of nutrient enrichment on biofilm metabolism (but see Lock et al., 1990; Guasch et al., 1995). Although some researchers have observed positive linear relationships between biofilm primary production and biomass (Bott et al., 1985; Morin et al., 1999), others have observed weaker relationships (Mulholland et al., 2001; Fellows et al., 2006a). Biofilm biomass and metabolism might respond differently to elevated nutrient concentrations for several reasons. First, because nutrient uptake kinetics fit a Michaelis–Menten relationship, primary production may not increase further as nutrient concentrations increase beyond the uptake capacity of the biofilm community (Bernot & Dodds, 2005; Earl et al., 2006). Second, the composition of algal and microbial assemblages may shift, with different taxa becoming more abundant at higher nutrient concentrations (Fairchild et al., 1985), altering responses to nutrient enrichment. Finally, physical characteristics such as flow (Francoeur & Biggs, 2006), light (Hill et al., 1995), and temperature (Marcarelli & Wurtsbaugh, 2006), and biological controls such as invertebrate grazing (Rosemond et al., 1993), are important structuring factors that may alter relationships between biofilm biomass and metabolic rates.

The goal of this study was to test the effects of nutrient additions on biofilm biomass and metabolism in the Upper Snake River basin (southeast Idaho, USA). To test how in-stream nutrient concentrations alter nutrient limitation of biofilms, we selected sites that differed in the magnitude and extent of human-caused impacts, and which therefore experienced a range of nutrient concentrations. Specifically, we ask if biofilm biomass and metabolism show similar responses to nutrient enrichment, and whether their responses to nutrient enrichment differ between more-impacted and less-impacted sites.

Materials and methods

Study area

The Snake River, a major tributary of the Columbia River, flows from its source in western Wyoming across southeast Idaho in the Snake River Plain. We used sites on the Snake River and on two of its tributaries, the Portneuf and Blackfoot Rivers. Two

bioassay locations on the Snake River were near Blackfoot (Upper Snake, Lower Snake; Table 1, Fig. 1). At the lower site, the Snake drains approximately 32,110 km^2 (elevation 1,340–4,180 m.a.s.l.). These two sites are relatively unimpacted with overall good water quality (Table 1). Two additional sites with overall good water quality were located on the Blackfoot River (Upper Blackfoot, Lower Blackfoot; Table 1, Fig. 1). The Blackfoot, which enters the Snake between the Upper and Lower Snake sites, drains approximately 2,670 km^2 at the lower site (elevation 1,420–2,980 m.a.s.l.). Six study sites were located on the Portneuf River, which flows into the Snake downstream of the Lower Snake site. The Portneuf is heavily impacted by urban, industrial, and agricultural land uses and contributes twice the total phosphorus (TP) load present in the Snake River upstream of its confluence with the Portneuf (Idaho Department of Environmental Quality et al., 2006). The Portneuf River is approximately 170 km long and drains a 3,445 km^2 basin (elevation 1,330–2,825 m.a.s.l.). It is subjected to direct and indirect nutrient loading, riparian and river channel degradation, and hydrologic regime alteration. Sites along the Portneuf were distributed as follows: three sites with increasing amount of agricultural land use impact (Portneuf river kilometer (PRK) 130, 95, 52), one at the beginning of a major urban area and below the largest tributary input (PRK 36), one below the urban area (PRK 21), and one downstream of both the urban area and a large influx of groundwater that contains high concentrations of inorganic N and orthophosphate from a major industrial site and adjacent agriculture (PRK 18; Campbell et al., 1992). The seventh site was on the largest tributary in the Portneuf River network, which joins the Portneuf at river kilometer 50. Marsh Creek (TRB 50) drains an area heavily used for agriculture and has high nutrient concentrations and a high sediment load (Fig. 1, Table 1). Channel structure at all sites was typical of mid-order, arid-land rivers, with wide channels, little riparian shading, and high light exposures.

Environmental monitoring

Environmental conditions at all of the study sites were monitored as part of regular water quality monitoring efforts. Discharge was directly measured at sites on the Portneuf using either a Flo-mate (Hach/Marsh-McBirney, Frederick, MD, USA) or a FlowTracker Handheld (SonTek/YSI, San Diego, CA, USA) flow meter, based on the method of Davis et al. (2001). All of the Blackfoot and Snake River sites corresponded with United States Geological Survey (USGS) gauging stations; therefore, Q at these sites was taken as a mean of the gauged daily discharge

Table 1 Water characteristics at the bioassay study sites during the NDS deployment periods in July–August and October–November

River	Site	July–August					October–November				
		Discharge (m^3/s)	Temp (°C)	Turbidity (NTU)	DIN (mg/l)	PO_4–P (mg/l)	Discharge (m^3/s)	Temp (°C)	Turbidity (NTU)	DIN (mg/l)	PO_4–P (mg/l)
Blackfoot	Upper	2.9	21.5	4.4	0.02	0.01	1.4	5.3	6.6	0.08	0.01
	Lower	16.4	21.8	NA	0.01	0.01	3.7	NA	NA	0.40	0.01
Snake	Upper	154.3	19.0	1.9	0.04	0.01	112.3	6.7	1.2	0.19	0.01
	Lower	58.1	19.8	4.1	0.02	0.01	97.9	7.9	6.5	0.13	0.01
Portneuf	PRK 130	–	–	–	–	–	0.1	4.5	4.4	NA	NA
	PRK 95	4.3	18.2	6.1	0.46	0.03	3.2	12.4	5.9	0.73	0.02
	PRK 52	0.6	19.8	30.3	0.32	0.01	4.0	8.8	11.9	0.64	0.01
	PRK 36	1.8	20.7	20.6	0.32	0.02	6.3	8.1	12.5	0.64	0.02
	PRK 21	2.9	20.6	14.2	0.25	0.01	6.7	7.9	14.4	0.67	0.06
	PRK 18	9.3	16.1	4.3	2.62	1.44	13.0	10.1	7.1	2.20	1.04
Marsh	TRB 50	1.5	20.7	14.1	0.46	0.03	2.4	8.5	11.8	1.10	0.03

– Indicates that bioassays were not conducted at that site during that time period; NA indicates that data is not available for that site on that date

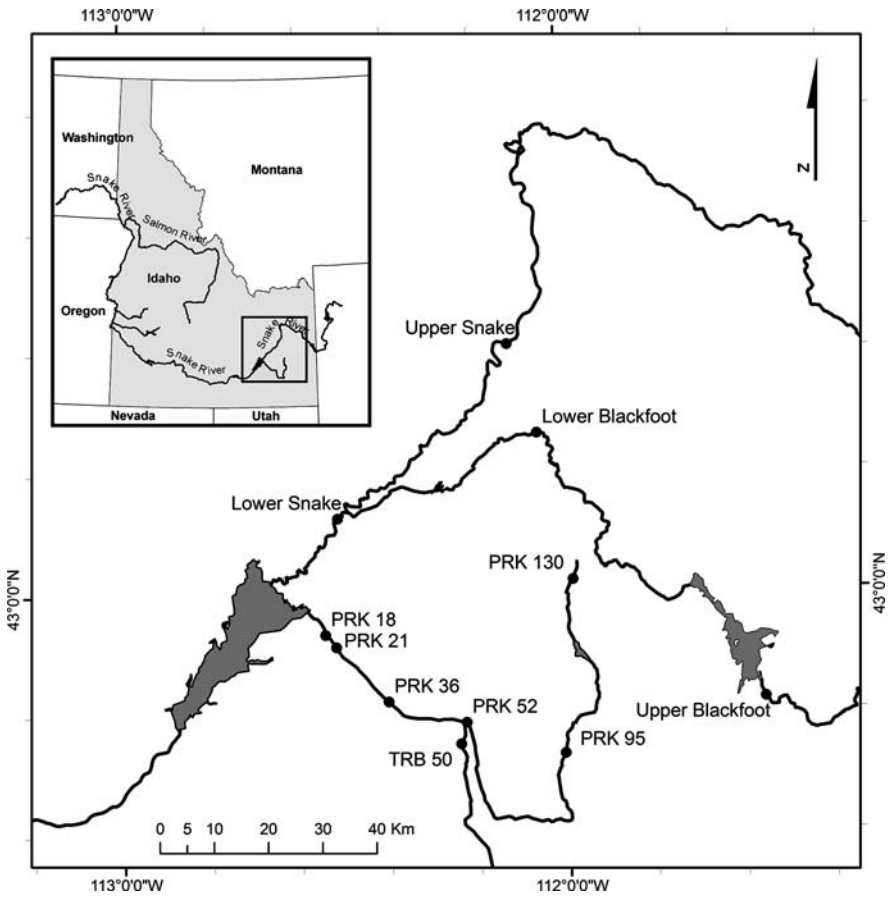

Fig. 1 Locations of the study sites where bioassay experiments were conducted on the Snake River and two tributaries, the Portneuf and Blackfoot Rivers. Inset shows the location of the study area in southeast Idaho, USA

(data available at http://waterdata.usgs.gov, site codes 13069500, 13060000, 13066000, 13063000). Water temperature and turbidity were recorded at 10-min intervals during the incubation period at a subset of the sites using YSI 6820 or 6920 multi-parameter sondes equipped with model 6560 combination temperature/conductivity probes and model 6136 turbidity probes (YSI Incorporated, Yellow Springs, OH, USA). At the remaining sites, water temperature was monitored at 15-min intervals using HOBO temperature loggers (Onset Computer Corporation, Pocasset, MA, USA). Temperature and turbidity are reported as mean daily values for the period of bioassay deployment. Dissolved nutrient samples were collected at the study sites monthly and analyzed for ammonium (NH_4–N), nitrate + nitrite (NO_3–N + NO_2–N), and orthophosphate (PO_4–P) using EPA standard methods (United States Environmental Protection Agency, 1983) by Energy Labs (Billings, MT, USA). For our study, we considered all three dissolved nitrogen species together as dissolved inorganic N (DIN). Water chemistry and sonde data from the Portneuf River were collected to support management plans and are available at http://www.portneufriver.org.

Bioassay design

Bioassays were conducted at all sites in summer (July, August) and autumn (October, November) 2006. On the Portneuf, bioassays were conducted in July, August, and late October, and on the Snake and Blackfoot, they were conducted in July and early November. Nutrient diffusing substrata (NDS) were constructed following the design of Gibeau & Miller (1989). Plastic 37-ml vials were filled with nutrient-enriched 2% agar and capped with 2.6-cm diameter fritted glass disks (Leco Corporation, St. Joseph, MI, USA). Four nutrient treatments (control, N-enriched, P-enriched, and N + P enriched) were used. Nitrogen and P were added to the agar as 0.5 mol N/l as $NaNO_3$ and 0.2 mol P/l as KH_2PO_4. At each site, six replicates

of each treatment were randomly distributed within one aluminum rack which was deployed on the riverbed (approximate depth 0.5 m) for 14 days.

At the conclusion of the incubation period, NDS vials were collected, placed into plastic bags, and transported on ice to the laboratory where they were frozen for storage. Within one month of collection, the glass disks were separated from the vials and analyzed for chlorophyll *a* (chl *a*) and ash-free dry mass (AFDM) using standard methods (American Public Health Association, 2005). Disks were extracted in methanol overnight, and the methanol was analyzed for chl *a* spectrophotometrically. Disks were then dried to a constant weight, oxidized at 500°C for 4 h, rewetted, and dried to a constant weight for AFDM determination following the methods of Davis et al. (2001). Autotrophic index (AI) was calculated as the ratio of AFDM to chl *a* (AFDM/chl *a*; Steinman et al., 2006).

Metabolism measurement

To test the relationship between metabolism and biomass, both were measured on the same substrata on the August and October 2006 study dates. To minimize transport and analysis time, metabolism was only measured for samples from the Portneuf River sites. The light–dark bottle technique was used to measure net primary production (NPP), community respiration (CR), and gross primary production (GPP; Wetzel & Likens, 2000). At the study site, rather than being placed in plastic bags, each substrate was placed into a separate 480-ml glass jar filled with river water. Replicates of each treatment were split equally between dark and light bottles, resulting in three light and three dark bottles for each treatment at each site. Light and dark bottle blanks containing only river water were also collected at each study site. Jars were transported to a greenhouse at Idaho State University where the metabolism incubations occurred.

At the greenhouse, initial dissolved oxygen concentration (DO_2) was measured in each jar using an Accumet AR40 DO_2 meter (Fisher Scientific, Pittsburgh, PA, USA). Jars were then distributed to minimize self-shading and incubated for 1.5–3 h. Photosynthetically active radiation (PAR) was monitored using a LI-COR Quantum sensor and LI-1000 data logger (LI-COR, Incorporated, Lincoln, NE, USA), and averaged approximately 350 μmol photons m^{-2} s^{-1} during the incubations. Air temperature in the greenhouse was maintained at 20–23°C. After the incubation period, final DO_2 concentration was measured and incubation time was recorded. Vials were then frozen and analyzed for biomass as described above. Metabolism metrics (NPP, CR, GPP) were calculated using the changes in light and dark bottle DO_2 concentrations, corrected for changes in blanks, using equations from Wetzel & Likens (2000). Metabolism rates were then scaled per unit chl *a* and AFDM biomass on the NDS.

Calculation of responses and statistical analysis

Differences in chl *a* and AFDM on control substrates were compared among sites and seasons using two-way analysis of variance (ANOVA, factors = site and season). To determine nutrient limitation status, responses of chl *a*, AFDM, GPP/chl *a*, and CR/chl *a* were analyzed at each site on each study date using a two-way ANOVA (factors = N presence/absence, P presence/absence). Limitation was classified following the criteria of Tank & Dodds (2003). Nutrient limitation by a single nutrient (N or P) was indicated when a significant response to only one nutrient was observed, but the interaction term was not significant. Primary limitation by either nutrient was indicated when a significant response to either N or P added alone was observed, and there was a significant interaction when both nutrients were added simultaneously. Co-limitation was indicated (1) if neither N nor P alone increased biomass, but there was a significant interaction between the two or (2) if both nutrients added individually increased biomass, with or without a significant interaction. No limitation was indicated by the lack of a significant response to any single or combined nutrient addition. ANOVAs were conducted in SAS version 9.1 (SAS Institute, Cary, NC, USA), and significance was considered at $\alpha = 0.05$.

Results

Seasonal and site variation in environmental characteristics, biofilm biomass, and metabolism

Discharge, temperature, turbidity, and nutrient concentrations varied strongly among sites and with

season. Discharge ranged from 0.1 to 154 m^3/s and varied seasonally between sites (Table 1). Discharge on the Blackfoot and Snake River sites tended to be lower during autumn than summer, except at the lower Snake site where discharge almost doubled during autumn. However, discharge increased at all Portneuf and Marsh sites except PRK 95 in autumn because of cessation of summer irrigation diversions. In general, discharges were similar at the Portneuf and Blackfoot sites, and 2–10 times higher at the Snake sites. Water temperatures were warmer during summer than autumn (Table 1). Temperature varied the least seasonally at PRK 18 and 95, likely due to groundwater inputs upstream of these sites. Turbidity was low at the less-impacted Snake and Blackfoot sites (1.2–6.6 NTU; Table 1). On the Portneuf, turbidity was also low at the less-impacted PRK 130 and 95 sites (4.4–6.1 NTU), and then increased at the more-impacted mid-river sites (PRK 52, 36, and 21). At these mid-river sites, turbidity was higher during summer (14.2–30.3 NTU) than autumn (11.9–14.4 NTU). Turbidity was lower at the PRK 18 site, again because of the groundwater inputs above this site. DIN concentrations tended to be greater during autumn than summer at all study sites, while there was no consistent seasonal pattern for PO_4–P (Table 1). DIN concentrations on the Portneuf were typically 1–2 orders of magnitude greater than those at the Snake and Blackfoot sites. On the Portneuf, DIN declined slightly (summer) or remained steady (autumn) between PRK 95 and PRK 21, and then increased 3–10-fold between the PRK 21 and PRK 18 sites at all sampling times (Table 1). PO_4–P concentration on the Portneuf showed similar patterns to DIN, but with much larger increases (17–140-fold) between PRK 21 and PRK 18 (Table 1).

Chl *a* concentrations were significantly greater in summer than in autumn across all sites, and generally greater at the Portneuf than the Snake or Blackfoot sites (two-way ANOVA, $F_{26,\ 131} = 22.9$, $P < 0.001$; Fig. 2A–C). Chl *a* concentration was greatest at the heavily impacted TRB 50 (Marsh Creek), and lowest at less-impacted PRK 130 (Table 2A). In addition, chl *a* concentration was significantly greater at the more-impacted PRK 52, 21, and 18 than at the less-impacted Upper Snake or Lower Blackfoot sites. A final group of sites (PRK 36, 95, Lower Snake, and Upper Blackfoot) had chl *a* concentrations that were intermediate and not significantly different from one another (Table 2A).

In contrast to chl *a*, AI (AFDM/chl *a*) was significantly greater during autumn than summer across all sites, indicating a more heterotrophic community in autumn (two-way ANOVA, $F_{26,\ 131} = 14.1$, $P < 0.001$; Fig. 2D–F). The among-site differences in AI were opposite to those of chl *a*, with the less-impacted PRK 130 having the greatest AI and the more-impacted TRB 50 and PRK 52 the lowest (Table 2B). Between these extremes were two groups of sites that were not significantly different from one another: the first contained Lower Blackfoot, PRK 95, PRK 36, and Upper Snake, and the second contained Lower Snake, PRK 21, and 18 (Table 2B). AI at Upper Blackfoot was intermediate between and not statistically different from these two groups. Differences in chl *a* and AI suggest that the most heavily impacted sites on the Portneuf (PRK 52, 18 and 21) and Marsh Creek (TRB 50) supported more autotrophic biofilms than other, less-impacted sites on the Portneuf (e.g., PRK 130 and 95), Snake, and Blackfoot, and that across all sites biofilms were more heterotrophic in autumn than in summer.

Although limited to sites on the Portneuf and Marsh Creek, GPP and CR on control NDS also varied among sites and between the two sampling dates (Fig. 3). A two-way ANOVA showed significant differences in GPP/chl *a* due to both season and site ($F_{12,\ 22} = 3.6$, $P = 0.005$), with higher rates in summer than in autumn (Fig. 3). In August, GPP/chl *a* was greater at PRK 95, 52, and 36 than at PRK 21, PRK 18, and TRB 50 (Fig. 3A), likely because of increased chl *a* concentrations at the three lower sites (Fig. 2B). Despite greater CR/chl *a* values across sites in autumn versus summer, the two-way ANOVA was marginally non-significant ($F_{12,\ 23} = 2.0$, $P = 0.07$), perhaps because of high variability and low replication at each site (Fig. 3A, B). However, higher CR/chl *a* during autumn was related to observations of more heterotrophic communities (higher AI) in autumn (Fig. 2F).

Nutrient limitation of biofilm biomass and metabolism

In the 27 NDS tests, nutrient stimulation or suppression of biofilm biomass was observed for chl *a* in 16 cases (59%) and for AFDM in 9 (33%; Table 3). For

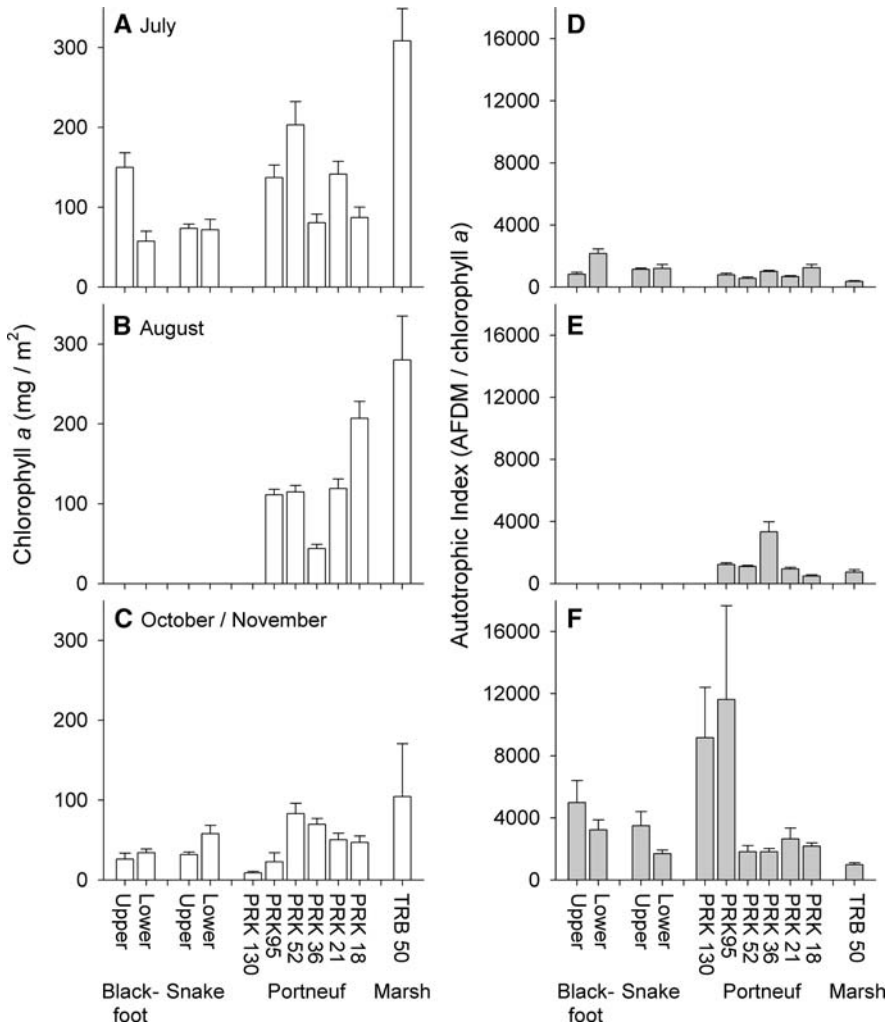

Fig. 2 Biomass on NDS controls expressed as chl *a* (**A**, **B**, **C**) and autotrophic index (**D**, **E**, **F**) ±SE in July (**A**, **D**), August (**B**, **E**), and October (**C**, **F**). Sites are arranged on the X-axis from upstream to downstream within each river (Blackfoot, Snake, Portneuf, Marsh)

all 27 tests, the most common chl *a* response observed was P suppression (18%), followed by N stimulation (15%), N and P co-stimulation (11%), primary N secondary P co-stimulation (11%), and N suppression (4%). The most common AFDM responses were N stimulation (15%) and N and P co-stimulation (15%), followed by primary N secondary P co-stimulation (4%). Phosphorus alone never stimulated or suppressed AFDM, and nutrient suppression of AFDM was never observed.

The biomass responses to nutrient enrichment differed among sites, and were more different in July than in October. In July, chl *a* at all four and AFDM at three of four less-impacted Snake and Blackfoot sites were significantly stimulated by N alone or N and P together. In contrast, on the Portneuf and Marsh Creek in July, chl *a* at one of six and AFDM at four of six sites was stimulated by N alone or by N and P together (Table 3). On the Portneuf and Marsh Creek in August, chl *a* was stimulated at four of six sites by N alone or by N and P together, while AFDM was never stimulated. Nutrient stimulation responses were rare in October/November across all the study sites (1 of 11 cases for chl *a*, 2 of 11 for AFDM). Suppression of chl *a* by P was observed only at three heavily impacted sites, PRK 21 (November), PRK 18 (July and August), and TRB 50 (July and November).

When nutrient additions had significant effects on metabolism (GPP and CR), they were driven by differences in biomass rather than by changes in

Table 2 Post hoc Tukey groupings of (A) chl *a* biomass and (B) autotrophic index (AI, AFDM/chl *a*) on control NDS across all study sites

A

Site	Tukey Groupings				
TRB 50	A				
PRK 52		B			
PRK 21		B	C		
PRK 18		B	C		
Lower Snake			C	D	
PRK 36			C	D	
PRK 95			C	D	
Upper Blackfoot			C	D	
Upper Snake				D	
Lower Blackfoot				D	
PRK 130					E

B

Site	Tukey Groupings				
PRK 130	A				
Lower Blackfoot		B			
PRK 95		B	C		
PRK 36		B	C		
Upper Snake		B	C		
Upper Blackfoot		B	C	D	
Lower Snake			C	D	
PRK 21			C	D	
PRK 18			C	D	
PRK 52				D	E
TRB 50					E

Tests were performed after significant differences were determined using two-way ANOVA (see text for details). Dashed lines illustrate possible grouping of sites

metabolic rates. When metabolism rates were scaled per unit area of the NDS (not normalized for biomass differences on the disks) there were no significant differences due to nutrient addition observed for either GPP or CR, except for P suppression of CR at PRK 36 in August (two-way ANOVA, $F_{3,\ 8} = 4.7$, $P = 0.03$). It should be noted that we measured a number of negative values for GPP; however, whenever these negative values were measured the standard errors were large and included zero. When normalized to chl *a* biomass of the biofilms, GPP was significantly suppressed by N on two of six occasions in August, simultaneously stimulated by P and suppressed by N on one occasion and suppressed by P and stimulated by N on one occasion, while CR was suppressed by N on one occasion and co-suppressed by N and P on one occasion. Almost all of these responses correspond to stimulation of chl *a* by N or N and P together (Table 4). Both biomass and metabolism responses to nutrients were rare in October. At this time, GPP/chl *a* was never affected by nutrients, and CR/chl *a* was suppressed by P on one occasion, stimulated by N on one occasion, and stimulated by P and suppressed by N on one occasion. None of these responses corresponded to significant biomass responses (Table 4).

Discussion

Biomass responses to nutrient addition across study sites

It is important to understand the interactions among nutrient supply, nutrient demand, and biofilm biomass and metabolism in streams, as biotic uptake can be an important mechanism that controls in-stream nutrient concentrations (Fellows et al., 2006a; Mulholland et al., 2006). Our results demonstrate that in southeast Idaho rivers, biofilm biomass responses to nutrient addition bioassays are highly variable both spatially and seasonally. Chl *a* was affected by nutrient additions in 59% of cases and AFDM in 33%. This response rate is typical of those observed in other stream systems; Francoeur (2001) found in a meta-analysis that chl *a* significantly responded to nutrients in 57% of nutrient bioassay experiments considered.

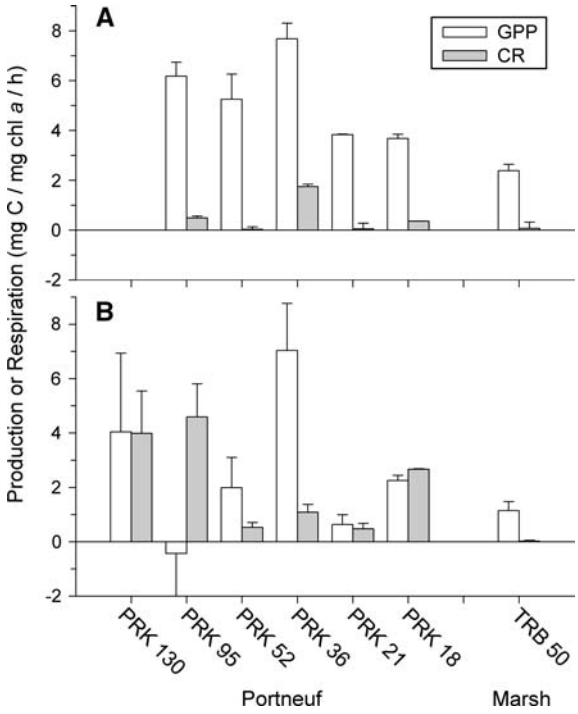

Fig. 3 Gross primary production or GPP and community respiration or CR, scaled per unit chl *a*, on NDS controls in (**A**) August and (**B**) October, ±SE. Sites are arranged on the X-axis from upstream to downstream within each river (Portneuf, Marsh)

The most commonly limiting nutrient for both biomass metrics was N, either alone or co-limiting with P (100% of AFDM and 63% of chl *a* responses). Despite the common paradigm that P limits algal biomass in freshwater ecosystems, N limitation of algal biomass was also the most frequent response noted in an interbiome stream nutrient limitation study (Tank & Dodds, 2003), and meta-analyses reveal frequent N and P co-limitation of algal biomass in both streams (Francoeur, 2001) and lakes (Elser et al., 1990, 2007). It should be noted that our study excluded macrophyte biomass and metabolism, which may comprise a significant portion of the primary producer biomass in certain reaches of the study rivers. Studies in the Portneuf River have demonstrated that rooted macrophytes are able to increase biomass and tissue P concentrations in response to increased P loads (Wilhelm, 2006).

Both water quality and nutrient limitation patterns appear to have been affected by the varying land use, particularly during summer. Less-impacted sites (Upper and Lower Snake, Upper and Lower Blackfoot, and Portneuf PRK 130 and 95) had lower DIN concentrations and turbidities, while more-impacted sites (Portneuf PRK 52, 36, 21, 18 and Marsh Creek TRB 50) had higher nutrient concentrations,

Table 3 Chl *a* and AFDM responses to nutrient enrichments at each study site and date, determined using two-way ANOVA

River	Site	Chl *a*			AFDM		
		July	August	October/ November	July	August	October/ November
Blackfoot	Upper	**1° N+ 2° P+****	–	NS	NS	–	**NP+***
	Lower	**1° N+ 2° P+****	–	NS	**N+***	–	NS
Snake	Upper	**1° N+ 2° P+****	–	NS	**N+****	–	NS
	Lower	**NP+****	–	NS	**N+****	–	NS
Portneuf	PRK 130	–	–	**N+****	–	–	**NP+****
	PRK 95	NS	**N+****	NS	NS	NS	NS
	PRK 52	NS	**NP+****	NS	**NP+***	NS	NS
	PRK 36	**N−***	**N+****	NS	**1° N+ 2° P+***	NS	NS
	PRK 21	**NP+***	**N+****	**P−***	**NP+****	NS	NS
	PRK 18	**P−***	**P−****	NS	NS	NS	NS
Marsh	TRB 50	**P−****	NS	**P−***	**N+***	NS	NS

* Indicates $P = 0.01$–0.05, ** indicates $P = 0.001$–0.01, *** indicates $P < 0.001$

Bold letters indicate the type of response, N = nitrogen, P = phosphorus, NP = nitrogen + phosphorus co-limitation

1° = primary limitation, 2° = secondary limitation, + = stimulation response, − = suppression response, NS indicates no significant response was observed, – indicates that bioassays were not conducted at that site during that time period

Table 4 Comparison of nutrient limitation in August and October at sites on the Portneuf River and Marsh Creek where both biomass and metabolic parameters were estimated, determined using two-way ANOVA

Site	August				October			
	Chl *a*	AFDM	GPP/chl *a*	CR/chl *a*	Chl *a*	AFDM	GPP/chl *a*	CR/chl *a*
PRK 130	–	–	–	–	**N+*****	**NP+*****	NS	NS
PRK 95	**N+****	NS	**N−*****	**N−***	NS	NS	NS	NS
PRK 52	**NP+****	NS	NS	NS	NS	NS	NS	**P−***
PRK 36	**N+****	NS	**N−***	**NP−*****	NS	NS	NS	NS
PRK 21	**N+****	NS	NS	NS	**P−***	NS	NS	NS
PRK 18	**P−*****	NS	**P+**, **N−*****	NS	NS	NS	NS	**N+***
TRB 50	NS	NS	**N+**, **P−***	NS	**P−***	NS	NS	**P+**, **N−***

* Indicates $P = 0.01$–0.05, ** indicates $P = 0.001$–0.01, *** indicates $P < 0.001$

Bold letters indicate a significant nutrient response and the direction of that response, N = nitrogen, P = phosphorus, NP = nitrogen + phosphorus co-limitation

1° = primary limitation, 2° = secondary limitation, + = stimulation response, − = suppression response, NS indicates no significant response was observed, – indicates that bioassays were not conducted at that site during that time period

turbidities, and chl *a* concentrations. In summer, the frequency of N limitation was higher at less-impacted sites than at more-impacted sites, and P suppression was observed only at the most-impacted study sites. More-impacted sites tended to have higher turbidity, which is positively related to light attenuation (Davies-Colley & Smith, 2001). Therefore, it is likely that more-impacted sites had lower overall light availability than less-impacted sites, which could also be related to the lower frequency of nutrient limitation responses at more-impacted sites (von Schiller et al., 2007).

Nutrient limitation responses were rare in autumn, even at the least-impacted sites. For example, in August biofilms at the Blackfoot and Snake River sites were very clearly limited by N or N and P together, while they were not nutrient limited in October. The lack of responses to nutrient additions in autumn could have been due to low water temperature, higher nutrient concentrations, and low light availability. Other studies have observed positive relationships between water temperature and algal biomass (Lamberti & Resh, 1983; Fellows et al., 2006b), and the presence of additional nutrients in the water column could suppress biomass responses on NDS. In the current study, temperature showed much more dramatic differences seasonally than spatially, despite the potential for human impacts to alter water temperatures (Paul & Meyer, 2001; Hagen et al., 2006), perhaps contributing to the large differences in nutrient limitation observed seasonally versus among sites. It is also possible that seasonal changes in light availability lead to lack of nutrient limitation responses by biofilms in autumn, as light availability has been linked to nutrient limitation responses in other studies (e.g., Tank and Dodds, 2003; von Schiller et al., 2007; but see Mosisch et al., 1999).

Despite the frequent responses of chl *a* to nutrient additions, metabolism responses seemed to be driven by changes in biomass rather than metabolic rates, which could be a result of the way we measured metabolism. First, although bottle incubations are easy to conduct and replicate, they are problematic because they eliminate water flow and subsequent gas exchange between the biofilm and overlying water (Hall et al., 2007) and can supersaturate easily with oxygen or carbon dioxide (Bott et al., 1997). We opted for bottle assays despite their limitations because we were interested in their potential as a management tool and in comparing a large number of nutrient treatments among many sites. Second, we measured metabolism after 14 days of deployment, when diffusion rates from NDS have decreased from initial rates and biofilms would be experiencing lower nutrient concentrations than when initially deployed (Rugenski et al., 2008). The effects of decreased nutrient-diffusion rates on biomass metrics should be less pronounced than on metabolism, as biofilms grow gradually and therefore integrate biomass responses to nutrient enrichment over time. In contrast, metabolic rates are instantaneous measurements and not integrated over time. However, at 14 days nutrients should

still be diffusing from the substrate, as other studies reported that nutrients are not depleted until after 21 days (Fairchild et al., 1985; Rugenski et al., 2008).

Assuming that our metabolic responses relative to control treatments would not be affected by the methodological limitations identified above, it is notable that in this eutrophic river, production and respiration responses to nutrient additions seemed to be mediated by increases in biomass, and not increases in overall biofilm metabolism. This could suggest that biofilm metabolism in this system is saturated with respect to nutrients at all of our study sites (e.g., Earl et al., 2006), or that some other factor is limiting primary production in our system. Biomass and metabolism of biofilm communities showed different responses to nutrient limitation when measured simultaneously. Therefore, biomass should not be considered a surrogate for biofilm metabolism in this ecosystem.

Evidence for autotrophic–heterotrophic competition in bioassay experiments

Phosphorus additions suppressed algal chl *a* in 18% of our experiments and only at the three most-impacted study sites (PRK 18, PRK 21, and TRB 50). Nitrogen suppression of algal biomass has been observed in headwater forest streams (Bernhardt & Likens, 2004), and P suppression of chl *a* has been observed in several other stream studies (Mosisch et al., 1999; Tank & Dodds, 2003). It is notable that although P additions decreased chl *a* in this study, there was never a concomitant decrease in AFDM, which includes algal biomass as well as heterotrophic biofilm components such as bacteria and fungi. It has been hypothesized that suppression of chl *a* could be due to competition between algae and heterotrophic bacteria for P, as the latter are superior competitors for this nutrient (Brussard & Riegman, 1998; Danger et al., 2007).

We hypothesize that at high nutrient sites, limitation of biofilms by N and P is alleviated and heterotrophic bacteria become limited by carbon supply, while autotrophic algae can fix carbon and therefore out-compete the heterotrophs. This could explain some of the differences in responses we observed between more- and less-impacted sites. For example, at our most-nutrient-impacted sites (PRK 18, PRK 21, and TRB 50), the AI on control NDS was significantly lower than at the other study sites, meaning the communities at those sites were more autotrophic, despite potential decreases in light availability due to increased water turbidity. In contrast, the AI generally increased at less-nutrient-impacted sites, suggesting that the communities at these sites were more heterotrophic, perhaps because of nutrient competition between algae and bacteria or fungi. Alternately, algal community composition at the most nutrient rich sites could have shifted to include more chl *a* rich species, which could have the same effect on the chl *a*-AFDM balance and resulting AI value. However, as we did not analyze community composition on our nutrient diffusing substrata, we do not have data to test this hypothesis.

The relative abundance of autotrophic and heterotrophic community elements varied seasonally, as chl *a* concentration decreased from summer to autumn while the autotrophic index increased. This suggests that biofilms are more dominated by heterotrophs in autumn than in summer. In addition, respiration by biofilm communities was greater in autumn than in summer, suggesting that the seasonal shift between autotrophs and heterotrophs translated into altered biofilm metabolism patterns. However, it is unclear what environmental factor led to this shift. Water temperatures were lower in autumn than in summer across all study sites, while DIN concentrations increased. Leaf fall also occurs during October and November in this area, which in streams is linked to increased organic C concentrations (Meyer et al., 1998) and increased heterotrophic activity (Bernhardt & Likens, 2002). It is all the more interesting that heterotrophic community responses occurred on the inorganic growth substrates used in the current study; organic growth substrates might further alter heterotrophic community responses (e.g., wood; Tank & Webster, 1998; Tank & Dodds, 2003). Further experiments with concomitant manipulations of nutrient and carbon supply are necessary to explicitly test our autotroph–heterotroph competition hypothesis in streams.

Conclusions

Our study demonstrated that biofilms in southeast Idaho rivers were primarily limited by N. Nutrient limitation was more frequent at sites with good water quality than at sites with poor water quality, and was

more common in summer than in autumn. In addition, responses of biofilm metabolism to nutrient additions were driven by changes in community biomass rather than changes in metabolic rates. While biofilm biomass is important for assessing river trophic state (Biggs, 2000), metabolism may be a more important metric for understanding the energy available for food web production (Lindeman, 1942) or the nutrient uptake capacity of the biotic community (Mulholland et al., 2006). A more thorough analysis of nutrient limitation in these rivers should include macrophyte communities, which comprise a significant portion of the primary producer biomass in certain river reaches.

One important implication of our findings is that heterotrophic and autotrophic biofilm components have the potential to respond differently to nutrient enrichments seasonally and across water-quality impacts. Future work should focus on this interaction among biofilm components and the commonly observed P suppression responses to gain a more complete understanding of how biofilms may respond to and buffer uptake and export of nutrient loads from nutrient-polluted rivers and watersheds.

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