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# Water quality, chlorophyll, and periphyton responses to nutrient addition in the Kootenai River, Idaho

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**Abstract:** During the past century, the Kootenai River, Idaho (USA), has experienced cultural oligotrophication following extensive levee construction, channelization, wetland drainage, and impoundment. A multiyear, whole-river nutrient-addition experiment was undertaken to mitigate these effects. The river was dosed with liquid agricultural-grade ammonium polyphosphate fertilizer (10-34-0) from June through September 2006–2010 to achieve an in-river total dissolved P (TDP) concentration of 3.0 µg/L. A fine-scale monitoring program included 8 sites over a 20-km reach (2 upstream control sites, one injection site, and 5 downstream treatment sites). Nutrient addition did not significantly increase N and P concentrations in the water column, but it significantly increased chlorophyll accrual rates and densities of edible green algae and diatoms. Nutrient addition significantly reduced  $\text{NO}_3^- + \text{NO}_2^-$  concentrations, atomic TN:TP ratios, and densities of inedible cyanophytes. Mean  $\text{NO}_3^- + \text{NO}_2^-$  values decreased along a downstream gradient below the nutrient-addition site, whereas chlorophyll accrual rate typically peaked immediately downstream from the nutrient addition site then decreased progressively downstream. Our study showed that nutrient addition is a useful river restoration technique for the Kootenai River.

**Key words:** nutrient addition, phosphorus, nitrogen, TN:TP ratio, water quality, chlorophyll accrual, periphyton, river restoration

N and P additions in aquatic systems can increase periphyton biomass, chlorophyll accrual rate, and the abundance and biomass of plankton and invertebrate communities (Kohler et al. 2008, Kohler and Taki 2010). Investigators also have reported significant increases in salmon production following nutrient addition in nutrient-depleted lakes and streams in British Columbia, Idaho, and Alaska (Johnson et al. 1990, Stockner 2003). The effects of reduced nutrient availability and biological production on naturally reproducing anadromous Pacific salmon populations have been described extensively in the literature (Schindler et al. 2003, Wipfli et al. 2003, Janetski et al. 2009), but anthropogenic oligotrophication also has reduced nutrient availability and biological production in rivers and lakes beyond the extent of marine-derived nutrient contributions (Ashley and Stockner 2003). Dam construction, wetland drainage, native fish population reduction or elimination because of over-fishing, habitat loss, and climate change are human activities that contribute to N, P,

and C removal from lakes, rivers, and streams (Stockner et al. 2000, Stockner and Ashley 2003). One example is the Kootenai River, a large altered montane river in British Columbia (Canada), Idaho, and Montana (USA). Extensive river diking, tributary channelization, impoundment, and the isolation and deforestation of >20,000 ha of floodplain habitat have contributed to decreased N, P, and C availability in the Kootenai River and the downstream Kootenay Lake (Canadian spelling) during the past century (Anders et al. 2002, Schindler et al. 2011). Impounded by Libby Dam, Koocanusa Reservoir is 32 km upstream from Libby, Montana, and acts as a large nutrient sink that retains an estimated 63% of total P and 24% of total N previously delivered downstream (Snyder and Minshall 2005). These effects have contributed to the decreased abundance of 6 native fish species in the river, with current estimates from 0 to 90% of historical numbers (KTOI and MFWP 2004). Snyder and Minshall (2005) conducted a study of autotrophic and detrital energy pathways in the Kootenai River

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and concluded that post-levee and post-dam P limitation probably was contributing to the reduced abundance of native fishes.

In response to this anthropogenic oligotrophy, a 5-y whole-river experimental nutrient addition study was implemented with the objectives of increasing nutrient availability and stimulating biological production in the Kootenai River. The process was assessed by: 1) monitoring nutrient concentrations (N and P) and TN:TP, 2) monitoring chlorophyll accrual and algal community composition; and 3) comparing results between upstream (control) and downstream (treatment) sites annually from June through September 2006–2010.

## METHODS

### Study area

The Kootenai River is a large, altered 7<sup>th</sup>-order river that drains a 42,000-km<sup>2</sup> watershed within mountainous boreal forestlands of British Columbia, Montana, and Idaho (Fig. 1). The basin is composed predominantly of folded, faulted metamorphosed sedimentary rock (Ferreira et al. 1992). The river is >700 km long and has a mean annual

discharge of 390 m<sup>3</sup>/s at the nutrient-addition site. The 20-km study area was in a lower canyon reach in a naturally constricted channel characterized by moderate bed gradient, gravel and cobble substrates, and intermittent bedrock outcrops (KTOI and MFWP 2004). This area was divided into 2 adjacent sections: an upstream 11-km control reach and a downstream 9-km treatment reach separated by the nutrient dosing site, which was in Idaho immediately downstream of the Idaho–Montana border (Fig. 1).

### Nutrient addition

An in-river concentration of 3.0 µg/L TDP (agricultural-grade liquid ammonium polyphosphate 10-40-0) was the annual target in the Kootenai River for 16 wk during the peak biological production season (1 June–1 October) from 2006 through 2010. Water-chemistry data showed the Kootenai River occasionally becomes N-limited in the late summer or early autumn (Hoyle 2003). Therefore, N fertilizer (liquid ammonium nitrate 32-0-0) was added as needed to maintain a minimum in-river TN:TP ratio of 20:1 if ambient NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> concentrations dropped <60 µg/L. Nutrients were added with a solar-powered gravity-flow dispensing system that included fertilizer storage tanks, a mixing head box, and dispensing pumps and flow monitoring meters as described by Holderman et al. (2009). Proper nutrient-dosing volumes and dilution rates were maintained to meet the 3.0 µg/L in-river TDP target by checking an on-site gage daily and adjusting dosing volumes accordingly.

### Sampling sites

Nutrients were added to the Kootenai River 81 river km (rkm) downstream from Libby Dam (Fig. 1). Eight sites were sampled over the 20-rkm study reach. Two control sites, KRF0 and KRF1, were 11 and 1 rkm upstream, respectively, and a 3<sup>rd</sup> control site KRF2 was just upstream of the dosing site. Five treatment sites (KRF3–11) were situated every 2 rkm downstream, beginning 1 rkm downstream from the dosing site (Fig. 1).

### Water chemistry

Total P (TP), total dissolved P (TDP), total N (TN), and NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> were sampled weekly at all sites at right-bank, mid-channel, and left-bank positions. Water samples were taken with a 2-L Van Dorn bottle within 4 m of the water surface and transferred to 250-mL bottles prerinsed with deionized water. Samples were stored on ice and shipped within 24 h to Aquatic Research Incorporated Laboratory (Seattle, Washington) for analyses. TP, TDP, TN, and NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> were analyzed with standard methods (APHA 2012). Samples were not filtered in the field or preserved because of the short holding times. Detection limits were

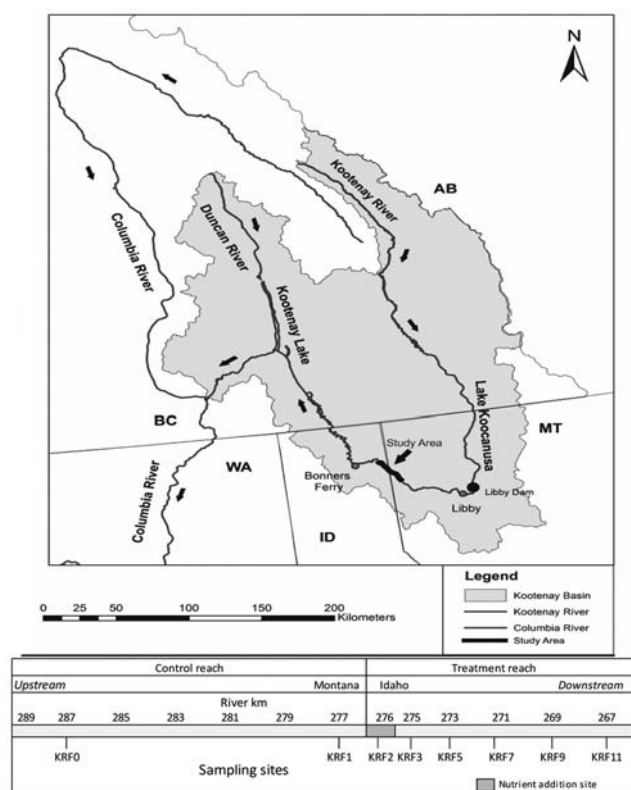


Figure 1. Study area and sampling sites used to evaluate nutrient addition in the Kootenai River, 2006–2010. AB = Alberta, BC = British Columbia, ID = Idaho, MT = Montana, WA = Washington.

2.0 µg/L for TP and TDP, 10.0 µg/L for  $\text{NO}_3^- + \text{NO}_2^-$ , and 50.0 µg/L for TN. Atomic TN:TP (mass:mass) ratios were calculated from mean TN and TP values (Ashley and Stockner 2003). Resulting empirical TN:TP < 10:1 indicated N limitation, values between 10:1 and 20:1 indicated colimitation, and values > 20:1 indicated P limitation (Redfield 1958, Ashley and Stockner 2003).

### Chlorophyll accrual

Two 30 × 30-cm cement tiles with 4 sections of 2.5-cm-thick Styrofoam® glued to their upper surfaces were placed on the substrate at each site to estimate chlorophyll biomass (µg/cm<sup>2</sup>) and accrual rate (µg cm<sup>-2</sup> d<sup>-1</sup>) using methods published by Perrin et al. (1987) and Bothwell (1989). Total chlorophyll (chlorophyll *a* + chlorophyll *b*) was sampled every 2 wk by collecting 2 Styrofoam punch cores per tile per sampling date with a circular metal corer (3.8 cm<sup>2</sup>; Bothwell 1989). Styrofoam substrate was replaced every sampling period to calculate accrual rates. Samples were removed from the corer and placed in labeled Whirl-Pak® bags, stored in brown plastic bottles, frozen at -20°C, and shipped to the University of Idaho Analytical Services Lab (Moscow, Idaho) for chlorophyll analysis (Wintermans and de Mots 1965).

### Algal community composition

Algal taxonomic composition was estimated from randomly selected, fixed-area (645.2 mm<sup>2</sup>) periphyton scrapes from rocks. One sample was collected per site per sampling date. Rocks were sampled randomly from near-shore areas at depths and velocities suitable for wading. Samples were preserved in 1% Lugol's solution by volume and sent to Aquatic Taxonomy Specialists (Malinta, Ohio) for taxonomic identification and enumeration. Soft-bodied periphyton cells were identified by viewing 300 cell-count wet mounts at 400× magnification. Bacillariophytes were identified in subsample burn mounts magnified up to 1000×. All enumerated algal specimens were identified at least to genus and were grouped as Chlorophyta (green algae), Bacillariophyta (diatoms), Cyanophyta (blue-green algae), or Other for analysis.

### Statistical analyses

A repeated measures analysis of variance (rmANOVA) was carried out on the nutrient and chlorophyll accrual responses. Response values for chlorophyll accrual were log(*x*)-transformed to meet distributional assumptions of the analyses. Analyses were used to evaluate the effects of sample site, time of sample, and the site × time interaction. Single degree-of-freedom contrasts were used to assess overall treatment effects between treatment and control sites. Values of ½ the laboratory detection limit were used for analysis when nutrient values were at or below detec-

tion limits. All nutrient and chlorophyll analyses were done separately with data from each year (2006–2010).

A contingency table with associated  $\chi^2$  tests of homogeneity was used to compare the overall consistency of algal community composition between treatment and control sites based on the 4 taxonomic groups listed above. Before  $\chi^2$  analysis, annual data from all sites were pooled within the control and treatment sites to assess treatment effects (nutrient addition) while retaining sufficient sample sizes for statistical analyses. All analyses were carried out using SAS OnlineDoc (version 9.2; SAS Institute, Cary, North Carolina) and statistical significance was assessed at the  $\alpha$  = 0.05 level.

## RESULTS

### Nutrients and chlorophyll

Mean TDP concentrations did not differ between treatment and control sites except during 2009, when TDP was significantly higher at treatment than at control sites (Table 1). Mean TDP concentrations ranged from 2.2 to 3.4 µg/L at treatment sites vs 2.0 to 4.8 µg/L at control sites (Table 2). Mean  $\text{NO}_3^- + \text{NO}_2^-$  concentrations were significantly lower at treatment than control sites (*df* = 1,16, *p* < 0.0001) during all years except 2007 (*df* = 1,16, *p* = 0.5563) (Table 1). Mean  $\text{NO}_3^- + \text{NO}_2^-$  values ranged from 97.4 to 140.7 µg/L at treatment sites vs 102.5 to 144.0 µg/L at control sites (Table 2). Mean TN:TP was significantly lower at treatment than at control sites during all years (Table 1), but the overall range of ratio values was nearly identical for the treatment and control sites and ranged from 20.4 to 44.1 over the duration of the study (Table 2). Chlorophyll accrual rates were significantly higher at treatment than at control sites during all years (Table 1) and ranged from 0.02 to 0.54 µg cm<sup>-2</sup> d<sup>-1</sup> at treatment sites and 0.007 to 0.061 µg cm<sup>-2</sup> d<sup>-1</sup> at control sites (Table 2). Up to 6-fold increases in chlorophyll accrual rates were observed at treatment vs control sites (Fig. 2). Immediately downstream from the nutrient dosing site,  $\text{NO}_3^- + \text{NO}_2^-$  values decreased significantly and chlorophyll

Table 1. *p*-values for contrasts between control sites (KRF0, KRF1, KRF2) and treatment sites (KRF3, KRF5, KRF7, KRF9, and KRF11) in the Kootenai River, 2006–2010. TDP = total dissolved P, TP = total P.

Year	Chlorophyll accrual	TDP	$\text{NO}_3^- + \text{NO}_2^-$	TN:TP
2006	0.0001	0.9343	0.0001	0.0001
2007	0.0001	0.8962	0.5563	0.0003
2008	0.0001	0.8106	0.0001	0.0001
2009	0.0001	0.0001	0.0001	0.0001
2010	0.0001	0.6326	0.0001	0.0001

Table 2. Mean ( $\pm 1$  SE) response values for control (KRF0, KRF1, KRF2) and treatment (KRF3, KRF5, KRF7, KRF9, and KRF11) sites in the Kootenai River, 2006–2010.

Year	Site	TDP ( $\mu\text{g/L}$ )	$\text{NO}_3^- + \text{NO}_2^-$ ( $\mu\text{g/L}$ )	TN : TP	Chlorophyll $a + b$ ( $\mu\text{g cm}^{-2} \text{d}^{-1}$ )
2006	KRF0	$2.177 \pm 0.207$	$102.457 \pm 2.316$	$44.140 \pm 1.44$	$0.008 \pm 0.001$
	KRF1	$2.041 \pm 0.192$	$107.641 \pm 2.097$	$39.927 \pm 1.307$	$0.012 \pm 0.003$
	KRF2	$2.451 \pm 0.237$	$105.619 \pm 2.387$	$24.835 \pm 2.708$	$0.009 \pm 0.003$
	KRF3	$2.153 \pm 0.178$	$104.783 \pm 2.483$	$25.961 \pm 1.042$	$0.165 \pm 0.026$
	KRF5	$2.448 \pm 0.201$	$103.763 \pm 2.426$	$26.043 \pm 1.445$	$0.113 \pm 0.019$
	KRF7	$2.328 \pm 0.252$	$99.074 \pm 2.269$	$25.336 \pm 1.169$	$0.290 \pm 0.080$
	KRF9	$2.222 \pm 0.179$	$97.367 \pm 2.553$	$44.140 \pm 1.112$	$0.136 \pm 0.030$
	KRF11	$2.191 \pm 0.162$	$98.122 \pm 2.386$	$26.466 \pm 1.163$	$0.168 \pm 0.030$
2007	KRF0	$2.587 \pm 0.189$	$143.973 \pm 2.645$	$33.011 \pm 1.516$	$0.061 \pm 0.012$
	KRF1	$2.652 \pm 0.210$	$140.668 \pm 2.210$	$31.401 \pm 1.473$	$0.059 \pm 0.016$
	KRF2	$3.321 \pm 0.179$	$141.466 \pm 2.123$	$20.438 \pm 1.969$	$0.052 \pm 0.009$
	KRF3	$3.335 \pm 0.165$	$140.672 \pm 2.133$	$22.140 \pm 0.703$	$0.435 \pm 0.094$
	KRF5	$3.286 \pm 0.209$	$139.396 \pm 2.047$	$21.741 \pm 0.786$	$0.536 \pm 0.111$
	KRF7	$3.282 \pm 0.196$	$138.844 \pm 2.117$	$23.733 \pm 0.937$	$0.362 \pm 0.068$
	KRF9	$3.223 \pm 0.179$	$136.944 \pm 2.067$	$21.577 \pm 0.829$	$0.322 \pm 0.062$
	KRF11	$2.957 \pm 0.167$	$136.404 \pm 2.226$	$22.615 \pm 0.868$	$0.388 \pm 0.070$
2008	KRF0	$2.053 \pm 0.118$	$134.054 \pm 3.835$	$37.392 \pm 2.388$	$0.025 \pm 0.003$
	KRF1	$2.130 \pm 0.161$	$130.268 \pm 4.031$	$36.313 \pm 2.508$	$0.012 \pm 0.002$
	KRF2	$2.484 \pm 0.199$	$132.127 \pm 4.053$	$21.605 \pm 2.617$	$0.010 \pm 0.002$
	KRF3	$3.082 \pm 0.256$	$129.697 \pm 4.065$	$21.791 \pm 0.854$	$0.064 \pm 0.010$
	KRF5	$2.592 \pm 0.202$	$123.539 \pm 3.702$	$22.585 \pm 0.995$	$0.063 \pm 0.006$
	KRF7	$2.906 \pm 0.217$	$124.391 \pm 3.418$	$20.809 \pm 1.078$	$0.110 \pm 0.020$
	KRF9	$2.864 \pm 0.186$	$121.851 \pm 3.479$	$22.696 \pm 1.104$	$0.045 \pm 0.006$
	KRF11	$2.647 \pm 0.137$	$119.265 \pm 3.599$	$21.447 \pm 1.433$	$0.074 \pm 0.017$
2009	KRF0	$2.002 \pm 0.130$	$120.274 \pm 3.676$	$31.683 \pm 1.352$	$0.026 \pm 0.004$
	KRF1	$2.138 \pm 0.12$	$117.726 \pm 3.647$	$29.584 \pm 1.462$	$0.008 \pm 0.001$
	KRF2	$2.410 \pm 0.150$	$118.076 \pm 3.389$	$27.266 \pm 1.416$	$0.011 \pm 0.002$
	KRF3	$2.764 \pm 0.199$	$119.174 \pm 3.412$	$20.618 \pm 0.848$	$0.081 \pm 0.007$
	KRF5	$2.738 \pm 0.143$	$115.083 \pm 3.624$	$20.498 \pm 0.954$	$0.139 \pm 0.033$
	KRF7	$2.783 \pm 0.170$	$112.929 \pm 4.022$	$20.625 \pm 0.818$	$0.097 \pm 0.013$
	KRF9	$2.812 \pm 0.189$	$110.731 \pm 3.857$	$21.640 \pm 0.825$	$0.035 \pm 0.004$
	KRF11	$2.843 \pm 0.157$	$109.176 \pm 3.869$	$21.786 \pm 0.831$	$0.063 \pm 0.007$
2010	KRF0	$2.278 \pm 0.194$	$127.791 \pm 2.504$	$43.280 \pm 1.783$	$0.008 \pm 0.001$
	KRF1	$2.300 \pm 0.210$	$123.318 \pm 2.405$	$41.539 \pm 1.976$	$0.007 \pm 0.001$
	KRF2	$4.771 \pm 1.088$	$123.631 \pm 2.416$	$31.562 \pm 2.245$	$0.007 \pm 0.001$
	KRF3	$3.436 \pm 0.423$	$120.278 \pm 2.238$	$24.334 \pm 0.940$	$0.141 \pm 0.023$
	KRF5	$2.762 \pm 0.340$	$116.509 \pm 2.468$	$25.183 \pm 0.969$	$0.139 \pm 0.051$
	KRF7	$2.707 \pm 0.263$	$117.116 \pm 2.503$	$24.700 \pm 0.974$	$0.054 \pm 0.006$
	KRF9	$2.724 \pm 0.203$	$115.404 \pm 2.624$	$24.709 \pm 0.870$	$0.022 \pm 0.002$
	KRF11	$2.282 \pm 0.201$	$113.233 \pm 2.837$	$24.941 \pm 0.847$	$0.079 \pm 0.017$

accrual rates peaked, followed by progressively decreasing downstream values for both metrics.

Bacillariophyte abundance was higher at treatment than control sites ( $df = 3$ ,  $p = 0.0382$ ) and accounted for 86 to

96% of the taxa. Edible algal forms (e.g., chlorophytes and bacillariophytes) were enhanced by up to 30 percentage points by nutrient addition, whereas the prevalence of inedible cyanobacteria decreased from 26 to as low as 6%,



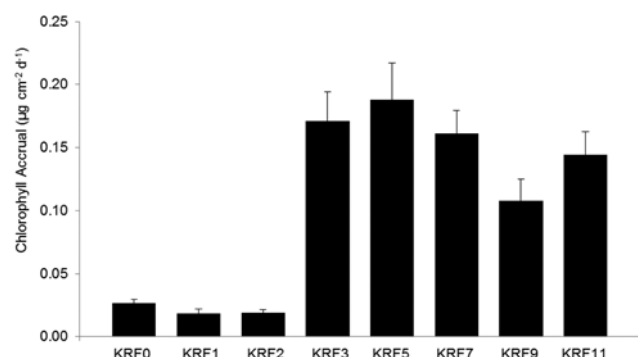


Figure 2. Mean (+1 SE) chlorophyll accrual, measured as chlorophyll *a* + chlorophyll *b*, at the control sites (KRF0, KRF1, KRF2) and treatment sites (KRF3, KRF5, KRF7, KRF9, and KRF11) in the Kootenai River, 2006–2010.

with a mean reduction in relative abundance from 17 to 5.5% following treatment (Fig. 3).

## DISCUSSION

Nutrient addition in the Kootenai River did not significantly increase TDP concentrations downstream of the injection location, except during 2009, when ambient N was low. Reductions and progressively decreasing downstream  $\text{NO}_3^- + \text{NO}_2^-$  values in the treatment reach probably resulted from rapid biological uptake, as seen in other nutrient-addition programs in ultraoligotrophic waters (e.g., Schindler et al. 2011). Slavik et al. (2004) attributed lower  $\text{NO}_3^-$  concentrations in the P-enriched reach of the Kuparuk River, Alaska, to increased biological uptake. Nutrient uptake was supported by observed increases in chlorophyll accrual rates, which were significantly higher in the treatment reach than in the control reach during all years of this study. The largest increase in chlorophyll accruals occurred in the 2<sup>nd</sup> season of the 3.0 µg/L P additions, whereas chlorophyll accrual decreased in subsequent seasons. This decrease in chlorophyll standing crop indicates a possible increase in benthic macroinvertebrate grazing pressure, as seen by Mundie et al. (1991) and Peterson et al. (1993). In the Kuparuk River, epilithic algae initially was limited by P availability, then by grazing benthic macroinvertebrates, followed by a shift to a moss-dominated periphyton community (Slavik et al. 2004). Increases in chlorophyll production following additions of limiting nutrients also were seen in southeastern Idaho rivers where biofilm responses varied spatially and seasonally and occurred in 59% of all instances (Marcarelli et al. 2009).

Nutrient addition produced favorable shifts in algal taxonomic composition, including increased densities of edible green algae and diatoms and reduced densities of inedible cyanophytes, that can contribute to foodweb enrichment (Naiman et al. 2012). Nutrient addition also reduced TN:TP from as high as 45:1 in the control reach

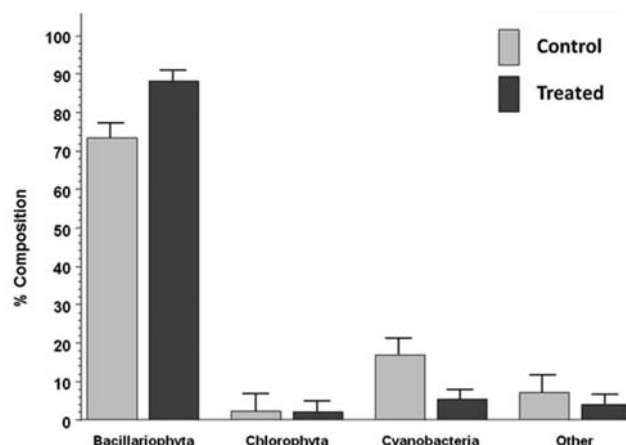


Figure 3. Mean (+2 SE) % composition of algal orders sampled in the Kootenai River at the control and treatment sites, 2006–2010.

to 20–30:1 in the treatment reach. This change constituted a substantial reduction in the degree of P limitation and partially counteracted the negative biological effects of anthropogenic oligotrophication.

The consistent, positive responses and the absence of undesirable outcomes lead us to recommend nutrient addition as a valuable restoration technique to enhance primary production in the Kootenai River and potentially in other anthropogenically oligotrophic rivers and streams, with the caveat that ecosystems respond to nutrient additions in highly specific ways (Artigas et al. 2013). Nutrient addition, coupled with other management tools, such as habitat restoration and foodweb analysis (Naiman et al. 2012), collectively can help restore threatened fish populations.

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