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RESPONSE OF ALGAL BIOMASS TO LARGE-SCALE NUTRIENT CONTROLS IN THE CLARK FORK RIVER, MONTANA, UNITED STATES[1]

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ABSTRACT: Nutrient pollution is an ongoing concern in rivers. Although nutrient targets have been proposed for rivers, little is known about long-term success of programs to decrease river nutrients and algal biomass. Twelve years of summer data (1998-2009) collected along 383 km of the Clark Fork River were analyzed to ascertain whether a basin-wide nutrient reduction program lowered ambient total nitrogen (TN) and total phosphorus (TP) concentrations, and bottom-attached algal biomass. Target nutrient and algal biomass levels were established for the program in 1998. Significant declines were observed in TP but not TN along the entire river. Downstream of the city of Missoula, TP declined below a literature-derived TP saturation breakpoint and met program targets after 2005; TN was below targets since 2007. Algal biomass also declined significantly below Missoula. Trends there likely relate to the city's wastewater facility upgrades, despite its 20% population increase. Upstream of Missoula, nutrient reductions were less substantial; still, TP and TN declined toward saturation breakpoints, but no significant reductions in algal biomass occurred, and program targets were not met. The largest P-load reduction to the river was from a basin-wide phosphate laundry detergent ban set 10 years before, in 1989. We document that nutrient reductions in rivers can be successful in controlling algal biomass, but require achievement of concentrations below saturation and likely close to natural background.

(KEY TERMS: algae; rivers/streams; monitoring; time series analysis; nutrients; environmental regulations.)

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INTRODUCTION

Increases in nitrogen and phosphorus entering streams and rivers have led to growing concern over associated effects on water quality and esthetic values (Dodds and Welch, 2000; Smith *et al.*, 2006; Paerl, 2009). Eutrophication problems occur in the Clark Fork River in Montana (Figure 1), and have prompted citizen complaints about the river since the 1970s (Watson, 1989a). Concerns included unesthetic levels of bottom-attached (benthic) algae, with potential negative effects on aquatic life (e.g., low dissolved oxygen) (Watson, 1989b). As a result of these concerns, data and analyses carried out in the 1990s were used to guide the establishment of nutrient targets for the river (Dodds *et al.*, 1997). In 1998, a basin-wide voluntary nutrient reduction program (VNRP) was established to improve river water quality (Tri-State Water Quality Council, 2005).

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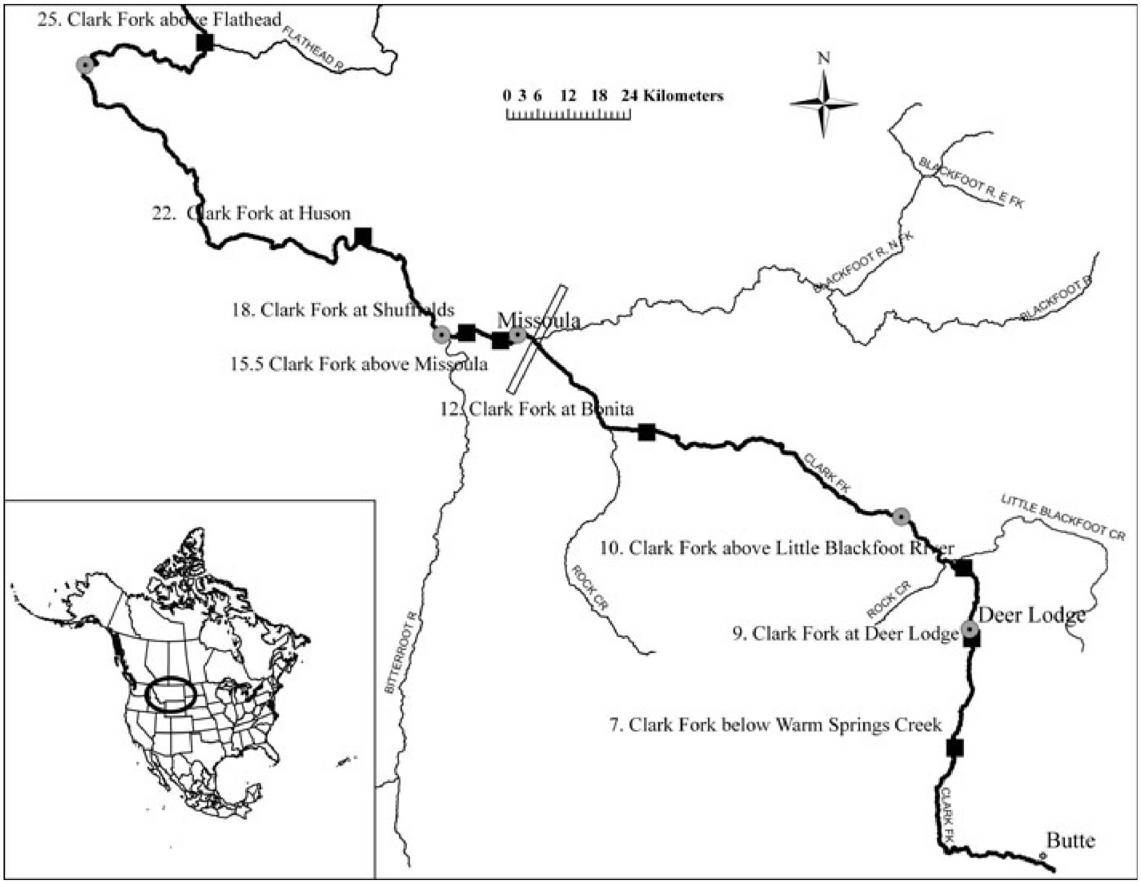

FIGURE 1. The Clark Fork River, Showing the Eight Study Sites (black squares) and Significant Tributaries. Gray dots are U.S. Geological Survey gage stations. The thick line mid-river demarks the upper and lower Clark Fork River as used in this article.

The VNRP goal was to minimize water quality and esthetic problems by reducing nitrogen and phosphorus loads to the Clark Fork River. Targets were derived for ambient water column nutrient concentrations and benthic algal chlorophyll *a* (Chl*a*) during summer, when stable low flows occur and problems with excessive algal biomass are most likely (typically July through September). Benthic algal biomass (Chl*a*) targets were set as summertime means of 100 mg/m^2, with a peak value of 150 mg/m^2. These biomass targets were subsequently demonstrated to match esthetic expectations for river recreation of the public majority living in the region (Suplee *et al.*, 2009), and are essentially the same as what is recommended to protect streams in New Zealand (120 mg Chl*a*/m^2) (Biggs, 2000). Nutrient targets were determined for total phosphorus (TP) and total nitrogen (TN) based on regressions linking water column nutrients to the specified levels of benthic Chl*a* (Dodds *et al.*, 1997). These concentrations have subsequently been supported by broader analyses (Dodds *et al.*, 2002, 2006). The targets were: 20 μg TP/l upstream of Missoula, 39 μg TP/l downstream of Missoula, and 300 μg TN/l throughout the river. Having first identified the TN target, the VNRP committee chose a TP target for the lower river that would achieve a balanced Redfield ratio (7:1 N:P by mass) (Redfield, 1958). For the upper river, a TP target was chosen that produced a higher N:P ratio (15:1, by mass) because of the concern that more phosphorus limitation was needed to control nuisance densities of the algae *Cladophora*, which is dominant there.

Major nutrient point sources were identified, and each of these sources set nutrient load reduction goals to reach the target concentrations. Most of the major actions to lower nutrient inputs were in place by 2004 (Table 1). In 2002, the state of Montana adopted the targets as water-quality standards for the river, although with modified boundaries for the TP criteria. These were the first numeric nutrient and algal biomass standards for a river in the state, and to our knowledge, some of the first such criteria adopted for a river in the world. Although targets were adopted as standards in 2002, for the purposes of this article we continue to refer to the original VNRP targets and boundaries.

Nutrients, and benthic algal Chl*a* and ash-free dry mass (AFDM) have been consistently monitored since

TABLE 1. Actions Taken to Remove Nutrients from the Clark Fork River and Their Effectiveness over the Period 1989-2005.

Nutrient Source	Action Taken	Sampling Site Immediately Below Action	Approximate Load Reduction Realized as of 2005 (kg/day)	
			TN	TP
Butte wastewater facility[1,2]	Constructed stormwater detention basins to reduce stormwater overflow to the sanitary sewers; reduced industrial loads; grew sod with effluent in summer (Note: New membrane bioreactor facility planned to be operational by 2015.)	7	−54	7
Deer Lodge wastewater facility[1]	Replaced old leaking sewer lines; developed a land application system for effluent to reduce direct July-September discharge to the river to zero (Note: Reductions occurred only up to 2008, as facility returned temporarily to direct discharge in 2008.)	10	11	2
Missoula County	Connected thousands of existing home septic systems to the central sewer[3]	18	35	1
Missoula wastewater facility[1]	Upgraded and expanded the facility to biological nutrient removal (BNR); operational late 2004	18	273	76
Smurfit-Stone Container Corporation[1]	Reduced nutrient additions to treatment systems; no direct discharge to river July-August (used storage ponds)	22	97	22
Basin wide	Phosphate laundry detergent ban emplaced in 1989[4]	All sites	0	121
Total load reduction to river (kg/day)			361	230

[1]Facility performance change was calculated as the average 1990-1993 daily nutrient load minus the 2005 daily load. Data sources were: appendix C in Ingman (1992), figures 2 through 6 in Tri-State Water Quality Council (2005), and data provided to the Montana Dept. of Environmental Quality (MT DEQ) by Land and Water Consulting (see Supporting Information Data S1, Table A).

[2]Butte's nitrogen load increased over the 1990-1993 to 2005 period.

[3]Per-household load reduction calculated as: ([daily home water-use volume] × [standard septic TN or TP effluent, mg/l]) − ([daily home water-use volume] × [average TN or TP effluent, mg/l, of the BNR facility]). Estimated that 2,400 existing home septic systems were connected to the BNR facility through 2005. We used 443 l/day/household, and septic effluent quality of 50 mg TN/l and 10 mg TP/l, with additional reduction in concentration due to passage through the soil of 15 and 90% for TN and TP, respectively. Data sources were: USEPA (2002), Tri-State Water Quality Council (2005), and Lowe *et al.* (2009). Load reductions shown are likely overestimated based on more detailed modeling underway (Eric Regensburger, Environmental Science Specialist-MT DEQ, February 23, 2012, personal communication).

[4]The P-ban went into effect May 1989. A 30% reduction in P was soon noted at the Missoula wastewater facility (Tri-State Water Quality Council, 2005). We calculated a 47% reduction in P concentration for Butte during the same period. Together, these translated to an average per capita mass of P from laundry detergent of 0.0012 kg/day based on 1990 census data for Missoula and Butte. The P-load reduction was then projected forward to the 2005 populations for Butte, Deer Lodge, Missoula, and Superior.

the VNRP began in 1998. Long-term datasets by which one can evaluate success or failure of nutrient reduction efforts on rivers are uncommon as far as we know. As such, the Clark Fork River dataset is important and was used to address the major goals of this article, which were to assess trends in total nutrients and benthic algal biomass – both river-wide and site-specific – especially at sites downstream from where major nutrient control efforts were emplaced.

METHODS

The reach of the Clark Fork River discussed here is located in western Montana and extends 383 km from its origins near Butte, Montana, to the confluence with the Flathead River (Figure 1). Summertime base-flow discharge volume increases over two orders of magnitude along the reach. Known or potential nutrient sources along the river include two communities each with populations over 30,000 (Butte and Missoula; 2010 census), a number of smaller communities, and various nonpoint sources (livestock and agriculture). This reach of the river had a single, rapidly flushed run-of-river impoundment just downstream of the Blackfoot River; however, the dam was removed and the river became free flowing in late 2010. Key changes in river water quality occur at the confluence of the Blackfoot River, which we use here to demark the upper from the lower Clark Fork River. The Blackfoot River increases July-September Clark Fork River discharge from ca. 8 to 48 m^3/s, lowers concentrations of TP by 43% and TN by 28%, and lowers river hardness by ca. 36% (1998-2009 data). This confluence is also an approximate transition point for benthic algal dominance, with *Cladophora* predominant in the upper Clark Fork River and a mixed assemblage of diatoms in the lower. The city of Missoula, the largest community along the reach (population 66,788 in 2010), is located downstream of the Blackfoot River confluence, upstream of the Bitterroot River confluence, and in between Sites 15.5 and 18. Clark Fork River summer discharge below the Bitterroot River confluence nearly doubles, after which small tributaries contribute discharge until the end of the reach where the summer discharge is ca. 110 m^3/s. Eight representative sites along this Clark Fork River reach had complete, continuous nutrient datasets from 1998-2009; increasing site number indicates that stations are further downstream (Figure 1). A subset of seven of the sites was targeted for summer benthic algae sampling over the same period (Table 2).

Nutrient samples were collected monthly during summer via grab samples from well-mixed portions of the river, and were analyzed using United States (U.S.) Environmental Protection Agency approved methods (Table 3). Total N was calculated as the sum of total Kjeldahl N (TKN) and $NO_3^- + NO_2^-$ (the latter two compounds analyzed concurrently from the same sample); by definition, TKN comprises organic nitrogen compounds and ammonia. Total N was determined in this way until 2008, after which TN was analyzed via persulfate digestion (Clesceri *et al.*, 1998). Direct measure of TN provided results comparable to TKN plus $NO_3^- + NO_2^-$ (comparative data not shown). During the study period, there were changes in field staff, analytical laboratories (Table 3), and electronic data storage protocols. Therefore, we carried out a very detailed (sample by sample) quality control of the entire dataset prior to undertaking any analyses. This included checking detection and reporting limits against original laboratory standard curves, and assuring that electronic data stored in different locations were reconciled. These quality control steps assured that the data were comparable and sufficiently precise and accurate for our analyses.

TABLE 2. Clark Fork River Sites Discussed in This Article.

River Locale	Site	Name	Latitude	Longitude	River km (from Columbia River confluence)	U.S. Geological Survey Gage Number	Algal Biomass Data
Upper	7	Clark Fork below Warm Springs Creek	46.188	112.7674	777.8	12324200	No
Upper	9	Clark Fork at Deer Lodge	46.382	112.736	745.1	12324200	Yes
Upper	10	Clark Fork above Little Blackfoot River	46.5052	112.7642	722.8	12324200	Yes
Upper	12	Clark Fork at Bonita	46.7177	113.5886	623.5	12324680	Yes
Lower	15.5	Clark Fork above Missoula	46.8643	113.9757	577.3	12340500	Yes
Lower	18	Clark Fork at Shuffields	46.8745	114.062	566.3	12340500	Yes
Lower	22	Clark Fork at Huson	47.0333	114.3429	530.1	12353000	Yes
Lower	25	Clark Fork above Flathead	47.3559	114.7826	395.3	12354500	Yes

Note: Associated U.S. Geological Survey gages shown.

TABLE 3. Analytical Methods Used to Quantify Nutrients and Algal Biomass at Sites Along the Clark Fork River.

Constituent	Method	Method Limit of Detection[1]	Reference (see also Literature Cited)
Total phosphorus (TP)	Persulfate digestion followed by SRP analysis	1 or 4 μg P/l	EPA 365.3 (also, Greenberg *et al.*, 1992)
Total Kjeldahl nitrogen (TKN)	Acid digestion followed by NH_4^+ analysis	100 μg N/l	EPA 351.2 (also, Greenberg *et al.*, 1992)
Total nitrogen (TN)	Persulfate digestion followed by nitrate analysis	5, 10, or 50 μg N/l	4500-N B or C (Clesceri *et al.*, 1998)
Nitrate + nitrite-N ($NO_3^- + NO_2^-$)	Cadmium reduction	2, 5, or 10 μg N/l	EPA 353.2 (also, Greenberg *et al.*, 1992)
Algal ash-free dry mass (AFDM)	Loss on ignition at 500°C	0.01 g/m^2 (hoop); 0.8 g/m^2 (template)	10200 I (Greenberg *et al.*, 1992)
Algal chlorophyll *a*	Spectrophotometric with acidification to correct for pheophytin	1 mg Chl*a*/m^2	Sartory and Grobbelaar (1984)

[1]Equivalent to the lower reporting limits given in the dataset used.

Field sampling for benthic algal Chl*a* and AFDM was accomplished via hoop sampling for heavy filamentous algal growth and template sampling for epilithic biofilms (Freeman, 1986; Watson and Gestring, 1996; USEPA, 2000, appendix B). For hoop sampling, a 30-cm diameter ring (710 cm^2 area) was tossed into the water and the filamentous algae within the confines of the ring collected. For template sampling – used when long filamentous algae were not present – all attached biofilm within a 25-cm^2 area (delineated using a template) was scraped from the top surface of a stone with a razor blade. Stones were cobbles 10-20 cm along the longest dimension. Both methods were used when both types were visually discernible. To collect samples without bias, field samplers walked upstream along a reach ≥100 m in length in a zigzagging manner, stopping about every 5 m to collect a sample. Field samplers restricted their movements and sampling to areas of the river 0.3-m deep; this was performed for safety (some parts of the river were too deep to wade) and to constrain sampling to a consistently monitored indicator zone. At each sampling point, samplers selected (without looking) a stone near to their foot or, if hoop sampling was appropriate, tossed the hoop in a random direction. Between 10 and 20 replicates (replicates equating to hoops, templates, or combinations thereof) were collected along the entire reach. All Chl*a* samples were held on ice in the dark until frozen (within 6 h of collection), then stored at −20°C until analyzed. Large filamentous samples were subsampled for Chl*a* analyses. Chl*a* was determined with hot ethanol extraction followed by spectrophotometric measurement (Sartory and Grobbelaar, 1984), and AFDM via standard methods (Table 3) (Greenberg *et al.*, 1992).

Mean monthly discharge data were taken from a U.S. Geological Survey (USGS) database (Table 2) (USGS, National Water Information System: http://waterdata.usgs.gov/nwis, *accessed* May 2011). River kilometer for each site was taken from DNRC (1984).

Data Analysis

The Dataset. The proportion of nondetects were: TP (0%); TN (6%); $NO_3^- + NO_2^-$ (20%). All nondetects for the TN measurements came from the TKN, with a single exception. The proportion of $NO_3^- + NO_2^-$ nondetects was slightly higher than desired for the method we used to process nondetects (converting them to ½ the lower reporting limit) (USEPA, 2006). However, $NO_3^- + NO_2^-$ data were always summed with concurrently collected TKN data to derive calculated TN values, and even in the very uncommon cases where TKN and $NO_3^- + NO_2^-$ were both below detection, $NO_3^- + NO_2^-$ would not be more than 9% of a calculated TN value (Table 3). Thus, we are confident that the slightly high proportion of nondetects for $NO_3^- + NO_2^-$ have not biased the results.

USGS gage locations do not correspond with the VNRP sampling sites, therefore VNRP data were associated with discharge data from the nearest USGS gage, with no major tributaries' confluences between the site and the corresponding gage (Figure 1; Table 2).

Data Reduction. We analyzed data by month (July, August, and September). We censored June nutrient data because June had much higher flows (due to snowmelt runoff effects) than July-September, and TP concentrations were greatly elevated due to suspended particulate materials (Froelich, 1988). As our goal was to understand water quality and algal biomass patterns during summer base flow, we concentrated on that period.

Nutrient data were processed using the following steps: At any given site, for any given year, and for any given parameter (e.g., TP concentration), data were reduced to a monthly average. First, all nondetect values were converted to ½ the recorded lower reporting limit. Then, quality control duplicates collected on the same day and at the same site were reduced to an average. Next, if sampling occurred at a site on more than one day during a month, the daily values were reduced to an average (nutrient sampling occurred on one to three different days each month). Finally, summer averages at a site were calculated as the average of the July, August, and September values processed as just described. Among the monthly nutrient concentrations (>1,000 data points), there were two extreme $NO_3^- + NO_2^-$ outliers (at Sites 18 and 22, August 1998) that were two orders of magnitude higher than the other $NO_3^- + NO_2^-$ data and would have influenced TN values; these were not included in statistical analyses.

Algae sampling occurred once per month. At a site (note that each ~100 m long reach = site), for any given month, the site replicates were reduced to an average. The term "maximum" as applied to algae (i.e., maximum Chl*a* or AFDM) is defined as following: during any given summer at any given site, the maximum algae value is the single greatest of any of the monthly Chl*a* or AFDM averages. There is only one month each summer representing the maximum (and only one maximum value per year). The month with the maximum Chl*a* was sometimes different from the month with the maximum AFDM.

Statistical Analyses. Statistical analyses were carried out with nonparametric methods, or parametric methods following $\log_{10}$-transformation of nonnormal data appropriately. First, we examined the relationship of station position and nutrients and algae using Spearman rank correlation (two-sided significance threshold = 0.05, MiniTab v16) (Conover, 1999). All benthic algae and nutrients decreased significantly with distance downstream; thus, the data indicated site-specific analyses or analyses that correct for the position effect should be performed.

We used the nonparametric Kendall family of tests (Helsel *et al.*, 2005) to examine nutrient concentration and algal biomass trends from 1998 to 2009, site-by-site, using two-sided tests with a significance threshold of $p < 0.1$ for rejecting trends. We selected this significance threshold for alpha error in order to keep beta error rates as low as possible. Beta error here represents the probability of declaring a truly significant trend as insignificant, a situation we wanted to keep to a minimum given the importance of ascertaining if nutrient reduction efforts may have had their intended effect. For this study, beta error is arguably more important than alpha error. The **n** in the analyses was fixed by site and relatively low (12-36 observations each); therefore, the remaining option for minimizing beta error was to increase alpha error (Ott, 1993).

Preliminary site-by-site analyses showed that total nutrients were influenced by monthly summer discharge at all sites (TP positively, TN negatively). Therefore, we employed a flow-adjusted approach (Hirsch *et al.*, 1982; Hipel and McLeod, 1994; Helsel and Hirsch, 2002) by using the seasonal Kendall test with locally weighted scatterplot smoothing (LOWESS) adjustment for one exogenous variable (mean monthly discharge). This method can discern temporal trends in a water-quality parameter that occur above and beyond that due to variation caused by temporal changes in discharge (Helsel and Hirsch, 2002). LOWESS smoothing was set at 0.5. In contrast to nutrients, algal biomass-discharge relationships were highly variable and no clear pattern could be discerned. Therefore, we did not use LOWESS adjustment for discharge for the algal temporal trends. For all seasonal Kendall tests, a season was equal to one month, and we report the p-values adjusted for serial correlation as the dataset is >10 years long (Hirsch and Slack, 1984). Maximum algal Chl*a* and AFDM were annual values (n = 12 years) and therefore the seasonal Kendall could not be used; we instead employed the Mann-Kendall test. The Mann-Kendall requires sample independence in order to assure accurate p-values (Helsel and Hirsch, 2002), so we checked for serial correlation in each site's dataset using the rank von Neumann test (USEPA, 2006).

Regional Kendall tests (Helsel *et al.*, 2005) were also undertaken and, for nutrients, LOWESS adjustment for discharge was performed in MiniTab v16. The regional Kendall examines whether or not, for any given parameter, the same directional trend is evident across all sites (e.g., is there a consistent pattern of TP decrease over time at all eight river sites?).

We also examined the changes over time, by site, for two time periods: 1998-2004 and 2005-2009. The two periods were chosen because most control measures were implemented by 2004, including a major wastewater facility upgrade in Missoula. Average values of nutrient or benthic algal biomass were plotted along with their associated 95% confidence intervals. Analysis of covariance (ANCOVA) was run in Statistica 6.0 (StatSoft, Tulsa, Oklahoma, USA), with site position as the continuous X-axis variable and the variable on the Y-axis separated into two categories – the before or after time periods. ANCOVA tests whether or not a factor (in this case, time period) has an effect on the Y-axis variable after removing the variance that is accounted for by the X-axis variable (in this case, position along the river). For this analysis, TN and TP were $\log_{10}$-transformed for normality, and the Durbin-Watson test run on the time-series data to check for serial correlation, as the serial correlation reduces the applicability of the F-distribution used to determine significance levels (Stewart-Oaten *et al.*, 1986; Neter *et al.*, 1989).

Breakpoint regression (piecewise regression) was used to find the breakpoint in the relationship between average summer nutrient concentration and average summer benthic Chl*a* (all sites). Ecological response to an environmental gradient is commonly nonnormal and nonlinear. Breakpoint regression shows where functional relationships change in the data and, within a broad range of values, where the peak occurs (Dodds *et al.*, 2010). The method minimizes the sum of square of errors with two lines to fit the data, and the breakpoint is where one relationship shifts to the other. The test determines whether adding the second line significantly increases the ability to fit the data distribution and indicates where the shift to the second line occurs. Breakpoint regression was carried out in Statistica 6.0.

RESULTS

Trends

Nutrients at each station were generally constant or declined over time. Five sites (9, 10, and 12, upper river; 18 and 22, lower river) had significant declines in summer TP after discharge adjustment. In contrast, discharge-adjusted TN showed no significant declines by site. Table 4 shows results for two key sites, 9 and 18; please see Table B in Supporting Information Data S1 for all sites. The regional Kendall analysis indicated TP significantly and consistently declined across all sites along the river ($p << 0.001$). However, TN did not show a significant river-wide change ($p = 0.175$). (Detailed regional Kendall results are available in Table C in Supporting Information Data S1.)

ANCOVA of the time period 1998-2004 *vs.* 2005-2009 also indicated overall nutrient decreases. The ANCOVA showed significant decreases in average TP ($p << 0.001$) and TN ($p = 0.022$) over time. However, there was evidence of serial correlation in the TN data at Sites 18, 22, and 25 (Durbin-Watson test statistic $d = 1.26$, 1.25, and 1.17, respectively). For TP, the serial correlation was only noted at one of the seven sites (Site 10) and d was 1.40, so the effect was minimal (critical value for declaring no serial correlation at 95% confidence for TN and TP was 1.42) (Neter *et al.*, 1989).

Temporal trends in algal biomass varied markedly between upper and lower river sites (e.g., Site 9 *vs.* 18; Table 4). In the upper river, there were no significant trends in algal biomass at any site. In the lower river (Sites 15.5, 18, 22, and 25), beginning at Site 18, maximum AFDM always declined significantly, and the other measure of algal biomass – Chl*a* – also declined significantly at the same sites. At Sites 18, 22, and 25, algal biomass trends were all downward, indicating an overall tendency for algal biomass to decrease at each site over time in the lower river. No serial correlation was noted in any of the maximum Chl*a* or AFDM datasets. The regional Kendall indicated mean and maximum algal AFDM significantly and consistently declined across all sites along the whole river (both p-values ≤0.003). However, algal Chl*a* (mean and maximum) did not significantly decline across all sites ($p = 0.813$ and 0.265, respectively).

Site 18 (lower river) showed some of the largest changes. Nutrient concentrations were generally above targets before nutrient controls and, in every case, nutrients and Chl*a* were higher there than what was observed at the site just upstream (Site 15.5) or at the next downstream (Site 22) (Figure 2). This longitudinal pattern was less evident after nutrient controls were emplaced (Figure 2). (Note: Data shown in Figures 2-5 has not been adjusted for discharge.) At Site 18 there was a significant decline over the 12 years in TP ($p = 0.035$), and a possible (but nonsignificant) decline in TN ($p = 0.152$) (Table 4; Figure 3). Site 18 also had significant declining trends in mean Chl*a* ($p = 0.036$), mean AFDM ($p = 0.056$), and maximum AFDM ($p = 0.064$). The LOWESS curve shows a notable downward step in TP concentrations in 2005, and a sharp decline in AFDM levels beginning the same year (Figures 3A and 3D).

TABLE 4. Kendall Tests of Trend Across Time for Nutrients and Algal Biomass for Two Example Sites: Site 9 (Upper River) and Site 18 (Lower River).

Test	Site	River Locale	Time Variable (X-axis)	Variable (Y-axis)	Exogenous Variable Controlled For	*n*	Kendall's Tau	*S*-value	*p*-Value	Conclusion
Seasonal Kendall with LOWESS	9	Upper	Month	TP	Mean monthly discharge (m^3/s)	36	−0.182	−36	**0.061**	**Trend (-); significant**
Seasonal Kendall with LOWESS	9	Upper	Month	TN	Mean monthly discharge (m^3/s)	36	−0.03	−6	0.866	Insignificant trend
Seasonal Kendall	9	Upper	Month	Average Chl*a*	n/a	33	0.162	26	0.157	Insignificant trend
Seasonal Kendall	9	Upper	Month	Average AFDM	n/a	33	−0.15	−24	0.336	Insignificant trend
Mann-Kendall	9	Upper	Year	Max Chl*a*	n/a	12	0.303	20	0.193	Insignificant trend
Mann-Kendall	9	Upper	Year	Max AFDM	n/a	12	−0.333	−22	0.150	Insignificant trend
Seasonal Kendall with LOWESS	18	Lower	Month	TP	Mean monthly discharge (m^3/s)	36	−0.364	−72	**0.035**	**Trend (-); significant**
Seasonal Kendall with LOWESS	18	Lower	Month	TN	Mean monthly discharge (m^3/s)	35	−0.241	−45	0.152	Insignificant trend
Seasonal Kendall	18	Lower	Month	Average Chl*a*	n/a	24	−0.341	−45	**0.036**	**Trend (-); significant**
Seasonal Kendall	18	Lower	Month	Average AFDM	n/a	24	−0.341	−45	**0.056**	**Trend (-); significant**
Mann-Kendall	18	Lower	Year	Max Chl*a*	n/a	12	−0.333	−22	0.150	Insignificant trend
Mann-Kendall	18	Lower	Year	Max AFDM	n/a	12	−0.424	−28	**0.064**	**Trend (-); significant**

Note: Bold values indicate that they are the cases where a statistically significant trend was detected.

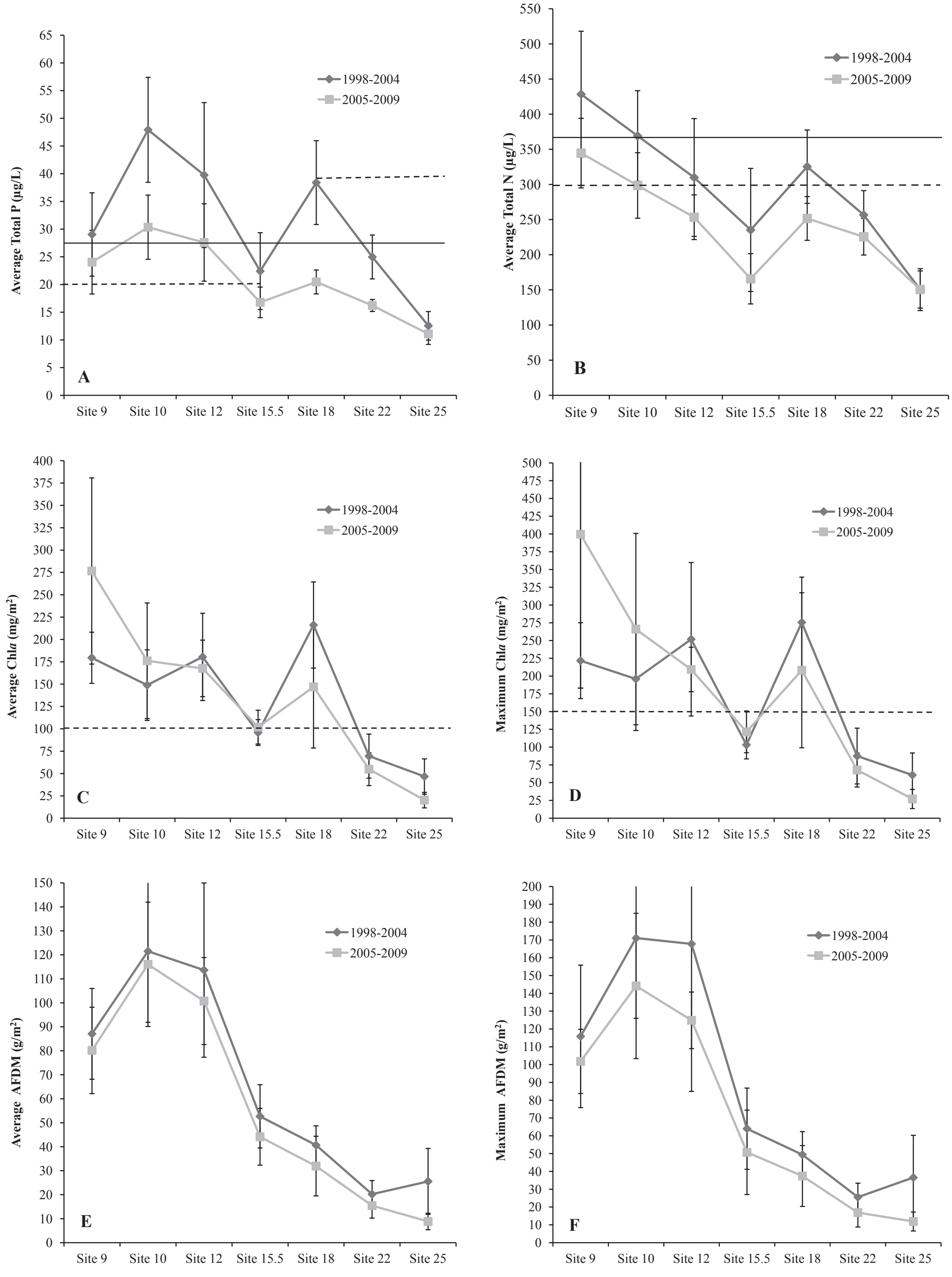

FIGURE 2. Mean Nutrient and Algal Biomass Trends for All Sites, 1998-2004 and 2005-2009. Error bars are the 95% confidence intervals. (A) Average TP; (B) average TN; (C) average Chl*a*; (D) maximum Chl*a*; (E) average AFDM; and (F) maximum AFDM. Also shown in A, B, C, and D are the VNRP summer targets (dashed lines) and, in A and B, the saturation breakpoint concentrations (solid line) from Dodds *et al.* (2006).

In contrast to Site 18, at Site 9 (upper river), there were no significant trends for benthic algae (Table 4). At a finer temporal resolution, the LOWESS curves show that the across-time trend for TP was inconsistent (first well above the target, then near it, then high again), for TN it was dome-shaped and usually

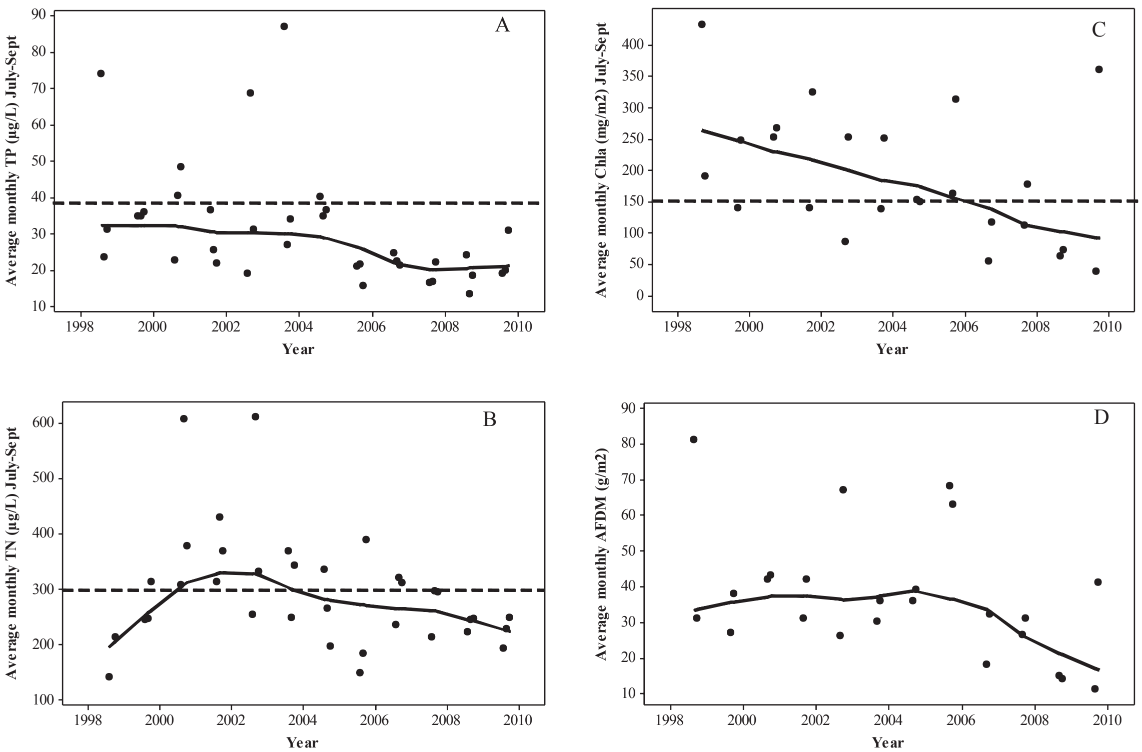

FIGURE 3. Nutrient and Algal Biomass Trends Across Time in the Clark Fork River at Site 18, Downstream of Missoula. (A) Average monthly TP (μg/l); (B) average monthly TN (μg/l); (C) average monthly benthic Chl*a* (mg/m^2); and (D) average monthly benthic AFDM (g/m^2). Solid lines are the LOWESS curve. Dashed lines are VNRP targets. Based on seasonal Kendall, downward TP trend is significant ($p = 0.035$); TN may be trending downward but is not significant (see Table 4). Also based on Kendall trend tests, average monthly Chl*a* and AFDM are significantly decreasing ($p = 0.036$ and 0.056, respectively).

above the target in the middle years, and for algal biomass it was mostly flat or increased in later years (Figure 4).

Saturation Thresholds and Achievement of Targets

Monthly averages were compared with VNRP targets as well as nutrient saturation thresholds. Piecewise regression between all-sites' data for TP and benthic Chl*a* indicated a significant breakpoint in the relationship at 24 μg TP/l (Figure 5). Although average TP concentrations in the upper river decreased, they still remained higher than VNRP targets and approximately equal to saturation breakpoint concentrations established from our data and by others (Dodds *et al.*, 2006). In the lower river below Missoula, all average TP concentrations after 2005 were below the VNRP target (39 μg/l), and control measures brought them near to or below the more restrictive *upstream* TP target (20 μg/l). Also in the lower river, average and maximum Chl*a* were at or better than the targets at Sites 15.5, 22, and 25. Between Sites 15.5 and 22, at Site 18, average TP concentrations were cut nearly in half following nutrient reductions from the wastewater facility, and average TN also showed concentration decreases (Figures 2A and 2B).

DISCUSSION

Trend analysis was useful in evaluating the varying effects nutrient reduction efforts have had along the river. In the upper river, declining TP is clearly indicated but algal biomass has not changed significantly. In the upper river at Site 9, algal biomass showed no indication of decrease and possibly even increased (Figure 4C). In contrast, both TP and algal biomass (Chl*a* and AFDM) significantly decreased at lower river Sites 18, 22, and 25 below the city of Missoula, and there are at least indications of a TN decline there as well. The regional Kendall tests support the notion that some nutrient (TP) and algal biomass (AFDM) levels are consistently declining along the entire river, and the ANCOVA indicated TP and TN both decreased after major nutrient control. The significant TN decline from the ANCOVA should be considered cautiously, however, as there was serial

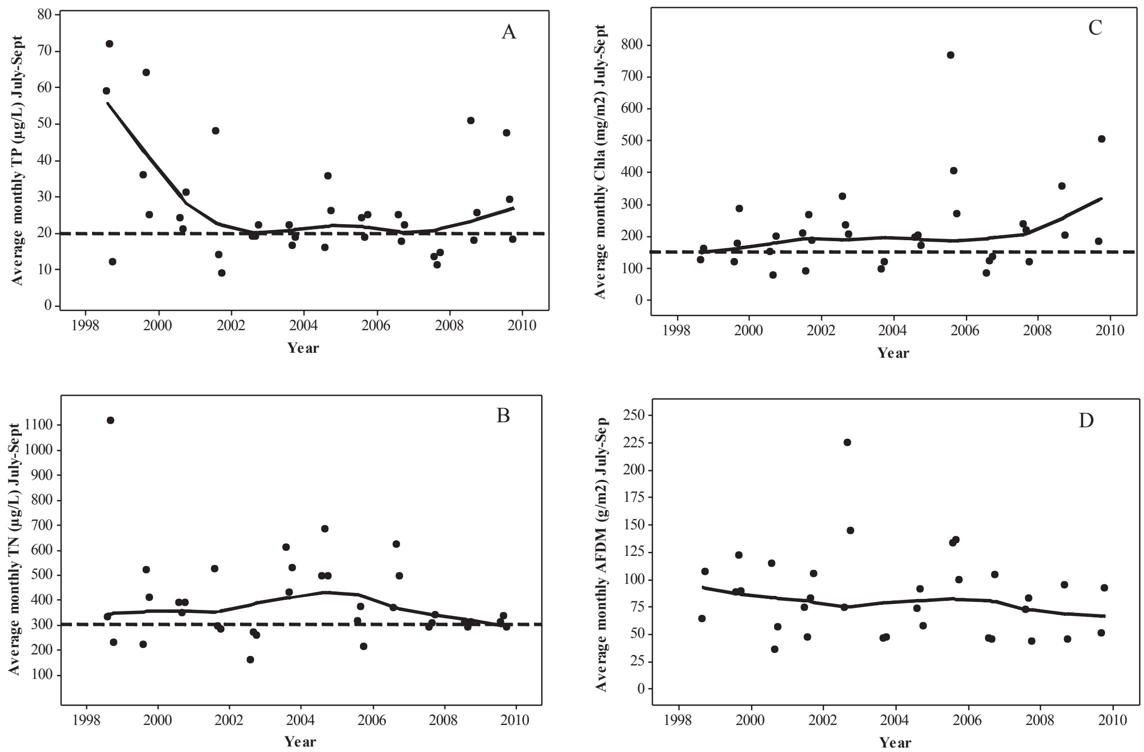

FIGURE 4. Nutrient and Algal Biomass Trends Across Time in the Clark Fork River at Site 9. (A) Average monthly TP (μg/l); (B) average monthly TN (μg/l); (C) average monthly benthic Chl*a* (mg/m^2); and (D) average monthly benthic AFDM (g/m^2). Solid lines are the LOWESS curve. Dashed lines are VNRP targets. Based on seasonal Kendall, downward TP trend is significant (p = 0.061); none of the remaining relationships shown are significant.

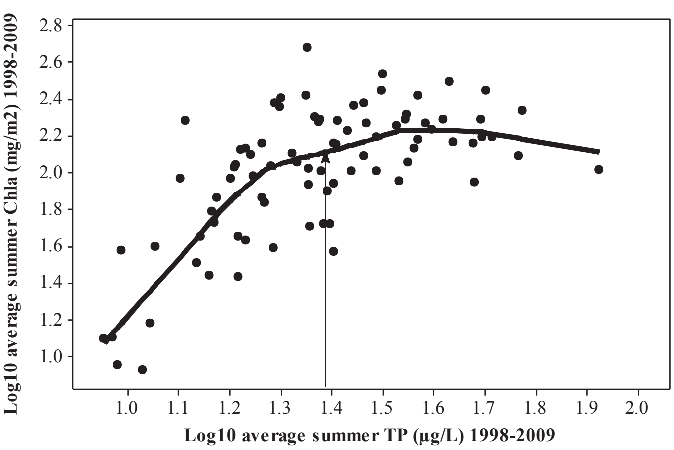

FIGURE 5. Relationship Between Average Summer TP and Benthic Chl*a*, All Sites, 1998-2009. Solid line is the LOWESS curve. The vertical arrow points to the breakpoint in the relationship (=24 μg TP/l).

correlation in the TN data at several sites and the test does not provide for discharge adjustment. Discharge-TN relationships were consistently negative and the significant decreasing TN trend per ANCOVA may merely reflect increases in summer discharge (flow-adjusted regional Kendall results support this notion). As it stands, we cannot conclusively determine whether nitrogen reductions were from increased river discharge or VNRP actions.

Analysis of nutrient-benthic algae regressions provides evidence for a saturating effect nutrients have on benthic algal biomass accrual in rivers and streams (Dodds *et al.*, 2002, 2006). Different methods (e.g., two-dimensional Kolmogorov-Smirnov, nonparametric changepoint analyses) give roughly similar indications of where functional relationships change in ecological data (Dodds *et al.*, 2010). Based on a large dataset of northern and southern temperate rivers and streams, saturation breakpoints for nutrient-benthic Chl*a* regressions were 27 μg TP/l (for mean and maximum Chl*a*), and 515 and 367 μg TN/l for mean and maximum Chl*a*, respectively (Dodds *et al.*, 2006). The TP data from the present study exhibited a similar breakpoint (24 μg TP/l) (Figure 5), but the TN data did not (benthic algae generally increased with all higher concentrations of TN, but the relationship was highly variable). These findings, all subsequent to the establishment of the VNRP targets, suggest that a single TP criterion of about 20 μg/l would be more likely to control algal biomass in the lower river as the current criterion (39 μg/l) is above phosphorus saturation.

Given the saturation breakpoints, it is not surprising that there was little effect of nutrient control on benthic algae in the upper river (Figure 2). At upper river Sites 10 and 12, TP concentrations in recent years are still above or hover near TP saturation breakpoints (Figure 2A). In contrast, at lower river Sites 15.5, 22, and 25, TP *and* TN breakpoint concentrations have mostly been met since 1998 and always since 2004. Site 18 (lower river just below Missoula) showed the greatest decrease in TP, where between 1998 and 2009 TP dropped significantly from well above to well below the saturation breakpoints (Figure 2A). Site 18 also showed the most significant algal biomass reductions. (Note in Figure 3C that the high Chl*a* value in 2009 at Site 18 was associated with 31 μg TP/l.) The existence of a breakpoint for TP in our data and the response of TP and algal levels to nutrient reduction efforts to date suggest that efforts to reduce or maintain phosphorus below breakpoint concentrations should continue.

Research conducted since the Clark Fork River nutrient targets were established indicates that the upper river TP target (20 μg TP/l) was set fairly close to natural background levels, but the TN target (300 μg TN/l) was substantially greater than natural background. Regional reference-stream data for the Middle Rockies ecoregion (Suplee *et al.*, 2007) and modeling based on levels in pristine rivers extrapolated to entire large watersheds (Western Forested Mountains ecoregion) (Smith *et al.*, 2003) indicate that natural background concentrations of TP and TN are expected to range from 10 to 18 and 85 to 190 μg/l, respectively. Thus, it may be possible to lower instream TN concentrations further, but achieving TP concentrations in the upper river below 20 μg/l may not be practical.

The dominant alga in the upper river, *Cladophora*, was expected to be difficult to control from the outset as previous analyses found no relationship between nutrients and its relative dominance (Dodds *et al.*, 1997). *Cladophora* has a patchy distribution, and high spatial variance during sampling makes it more difficult to detect significant changes. Further, upper river sites have very hard water in summer (ca. 214 mg $CaCO_3$/l), which is preferred by *Cladophora* (Whitton, 1970). Just upstream of Site 15.5, the Blackfoot River lowers summer hardness in the Clark Fork River by 36%. It is at this point in the river that *Cladophora* dominance diminishes, likely the result of hardness and nutrient declines. Upper river Site 12 recently achieved the target for TN, but it is still hard to state conclusively whether it will (or will not) achieve the algal biomass targets if/when the 20 μg TP/l target is reached. At nearby Site 10, benthic algae biomass appears to be tracking TN (data not shown), and earlier work on the upper Clark Fork River shows *Cladophora* tracks soluble nitrogen concentrations and may be nitrogen-limited (Lohman and Priscu, 1992). Upper river sites tend to give mixed signals, some suggesting stronger P limitation (Site 9 sometimes, and Site 12), others stronger N limitation (Site 10); vacillation over time and space in nutrient limitation of inland waters has been described by others as well (Gibson, 1971; Lewis *et al.*, 2011). Indeed, broader-scale analyses show that productivity in flowing water (and other aquatic systems) is often co-limited and therefore substantially increased when nitrogen and phosphorus (as opposed to just one of them) are enriched above natural background (Francoeur, 2001; Elser *et al.*, 2007; Lewis *et al.*, 2011). If these upper river sites achieve the TP but not the algae target, the TN target could possibly be lowered further as the TN target is still above natural background.

Across the whole river, at those sites where 20 and 300 μg TN/l are being achieved (e.g., Sites 15.5 and 18), the algae targets are also usually achieved. In a river system like this, adaptive management makes the most sense; that is, a process whereby targets are established, controls emplaced, and results monitored and then, at some future point, the targets reevaluated, as has been done here. Indeed, Butte's plans for a major facility upgrade by 2015 suggest that it is advisable to revisit the conditions and targets some 5+ years in the future, particularly the conditions and targets in the upper river. If the improvements observed below Missoula are any guide, one might anticipate substantial changes in the upper river due to Butte's upgrade.

We found the relative importance of different management actions to reduce nutrients quite striking. Nearly 10 years before the VNRP program, the phosphate laundry detergent ban had already greatly reduced P loading and was, in retrospect, the singlemost effective means of reducing phosphorus in the basin (Table 1). The ban had the added advantage of reducing P loads in a more uniform manner along the river. This was followed in importance 15 years later by Missoula's facility upgrade (to enhanced biological nutrient removal via modified Johannesburg Process). Missoula is the largest community along the river and roughly twice as populous as Butte. On the nitrogen side, Missoula's 2004 facility upgrade was the most important basin-wide action, although process changes instituted at a single industrial facility made very large N-load reductions as well.

The nutrient reduction work in Missoula is clearly reflected in water-quality downstream of the city. From 1998 to 2009, conditions at Site 18 moved from above to below the nutrient targets and closed in on the algal targets (Figure 3). Data from 2010 from Site 18 indicate that the decline in algal biomass continues on track (August and September averages were 66 and 139 mg Chl*a*/m^2, respectively) (Vicki Watson, 2010, University of Montana, unpublished data). The U.S. census shows the Missoula population grew over 20%

in the previous decade. During this time, the city connected unsewered septic systems to the upgraded wastewater facility, achieving a net decrease of about 3,000 septic systems and some notable load reductions (Table 1). The marked step down in TP concentrations after 2004 (Figure 3A) was a result of the upgrade and, to a lesser extent, the septic system hook-ups (Table 1). Although the upgrade to the wastewater facility provided larger N- and P-load reductions to the river than did septic hook-ups, looking forward, the septic hook-ups have put the city in a good position to provide better effluent treatment in the future as the need arises.

Our findings are consistent with those on the Bow River (Alberta, Canada). There, over an eight-year period, phosphorus and later nitrogen-removal technologies were instituted at major municipal wastewater facilities, and benthic algae declined significantly at locations downstream of the facility discharge points (Sosiak, 2002). Our findings are also consistent with larger-scale analyses showing that population density and the proportion of urban areas are positively correlated with nutrient concentrations in the Western Forested Mountains ecoregion (Dodds and Oakes, 2004). The latter study also shows that the proportion of land in pasture or livestock range land correlates with higher nutrient levels in rivers and streams, suggesting that efforts to control nutrient inputs to the Clark Fork River should continue to consider these potential sources.

There could be additional benefits to controlling nutrients in the Clark Fork River besides lowering benthic algal biomass, and nutrient control could help restore biotic integrity based on heterotrophic components of this system. Heterotrophic state of rivers and streams is an important component of their ecology, and nutrient additions can alter both the autotrophic state (algal or macrophyte photosynthesis) and the heterotrophic state (rates of bacterial metabolism of allochthonous carbon) (Dodds, 2006). As such, nutrients have been related to fish and invertebrate diversity in rivers and streams in the Midwest U.S. (Wang *et al.*, 2007; Evans-White *et al.*, 2009); presumably such relationships occur in other regions and likely apply to the Clark Fork River.

In conclusion, trend analyses showed that nutrient reduction efforts along the Clark Fork River were successful in significantly reducing TP concentrations basin-wide between 1998 and 2009. Although significant P-load reduction during this period was attributable mainly to wastewater facility changes and upgrades, interestingly, the action that reduced P loads to the Clark Fork basin the most was the emplacement of a phosphate laundry detergent ban 10 years earlier, in 1989. In contrast to TP, significant basin-wide reductions in TN did not occur between 1998 and 2009; however, the Missoula wastewater facility upgrade did provide the single largest reduction in N-load to the river. From 1998-2009, reductions in algal biomass and nutrients were clearly evident in the lower river below Missoula where nutrients were reduced below target concentrations and saturation breakpoints, and we recommend that the TP target in the lower river be lowered to about 20 μg TP/l as well (to assure TP remains below saturation). Consistent reduction in algal biomass down to the targets was not achieved in the upper river, where *Cladophora* dominates and where nutrient levels were not reduced well below saturation breakpoints and program targets were not consistently achieved. Despite human population growth in the watershed and in Missoula, it appears that nutrient reduction efforts have kept water-quality conditions from deteriorating and have even produced improvement at some sites. The research indicates that establishing nutrient targets below saturation breakpoints and fairly close to natural background levels is necessary to maintain benthic algal biomass at levels that people find acceptable for river recreation.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary materials mentioned in the text, specifically data used to calculate nutrient loads (Table A, which supplements Table 1 of the article), Kendall trend results for all sites (Table B, which supplements Table 4 of the article), and all regional Kendall results (Table C) are available as part of the online article.

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