Episodic loadings of phosphorus influence growth and composition of benthic algae communities in artificial stream mesocosms

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Abstract. Phosphorus (P) is an essential macronutrient for algal communities, but in excess can exacerbate stream eutrophication. However, P loadings to streams vary temporally from continuous to episodic as a result of inputs from point and non-point sources, respectively. P loading pattern can thus alter the temporal availability of P and may influence effects of P enrichment on algal communities. We assessed how P loading pattern influences algal biomass and composition by conducting a 29-day P enrichment experiment in nine artificial streams exposed to either: (1) continuous P enrichment; (2) episodic P enrichment, or; (3) no P enrichment. P enrichment increased algal biomass accrual, but peak biomass did not differ between continuously and episodically enriched treatments. Maximum absolute growth rates were also comparable between P enriched treatments. However, episodic P additions sustained elevated rates of biomass accrual, whereas absolute growth rates in the continuously enriched communities declined towards the end of the experiment. P enrichment resulted in comparable increases in relative abundance of chlorophytes and decreased proportions of bacillariophytes and charophytes in algal communities for continuously and episodically enriched treatments. However, composition of bacillariophyte (diatom) assemblages differed significantly among all P enrichment treatments in accordance with species autecological attributes for P. Our results demonstrate that episodic and continuous P enrichment may augment algal biomass similarly. Yet, P loading pattern regulated the composition of algal communities. Thus, remedial management strategies for the control of nuisance algae production may require focus on the predominant source of P to streams. Finally, species specific responses of diatom assemblages to P enrichment and associated loading patterns suggests this taxonomic group may have potential as diagnostic indicators for identifying the presence of key nutrient sources associated with eutrophication of stream ecosystems.

Key words: algae; diatoms; eutrophication; phosphorus; loading pattern

1. Introduction

Phosphorus (P) enrichment is regarded as a common cause of freshwater eutrophication (Correll, 1998), and many past studies have reported P limitation or co-limitation of benthic algae in streams (Elser et al., 2007; Francoeur, 2001). In P limited systems, increased biomass (Bowman et al., 2005; Biggs, 2000; Chételat et al., 1999) and growth rates (Fellows et al., 2006; Rier and Stevenson, 2006) of benthic algal communities have been positively associated with P concentrations and bioavailability. Similarly, the relative abundance of algal species can differ across stream P gradients (Black et al., 2011; Stevenson et al., 2008; Taylor et al., 2018). For example, Stevenson et al. (2012) reported an increase in nuisance green algae with greater total P concentrations among 70 streams in the US Midwest. In response to findings that benthic algal communities respond readily to changing P concentrations, watershed managers have regularly adopted P concentration guidelines to mitigate excessive algal growth in streams (Evans-White et al., 2013). However, temporal variation in stream P loading from human activities has the potential to confound benthic algae-P relationships and result in unanticipated ecological consequences (Jarvie et al., 2013).

Temporal variability in P loading to streams can depend on the type and intensity of human land use (Edwards and Withers, 2008). In undisturbed catchments, P loadings to streams are low and primarily associated with precipitation events that result in soil erosion and mineral weathering. Runoff events can transport particulate P to streams and cause temporal variation in the total P load, although the bioavailable fraction of P often varies minimally despite changes in discharge (Meyer and Likens, 1979). In contrast, anthropogenic P loadings can substantially increase the quantity of P in streams (Mainstone and Parr, 2002; Withers and Jarvie, 2008). Point sources (e.g., wastewater discharge) typically input enriched loads of bioavailable P from discrete locations with minimal temporal variability (Jarvie et al., 2006). Conversely, P loads from non-point sources (e.g., urban and agricultural lands) are derived from the landscape and transported to streams through episodic runoff events (Mainstone and Parr, 2002; Withers and Jarvie, 2008). Consequently, inputs of P from non-point sources exhibit substantial temporal variation in particulate and bioavailable P loads (Sharpley et al., 2001). Streams draining human-modified catchments can therefore receive enriched loads of P from two sources each with a distinct loading pattern: continuous inputs from point sources or episodic inputs from non-point sources. Effective management of stream eutrophication in catchments with mixed anthropogenic land uses thus requires information on the relative contributions that point and non-point sources have on stream P enrichment. However, few studies have considered how the loading pattern of P impacts stream eutrophication.

Traditionally, loads associated with episodic P enrichment have not been considered ecologically relevant to stream ecosystems (Stamm et al., 2014; Withers et al., 2014). However, many algae taxa are capable of assimilating excess P during periods of P surplus (i.e., “luxury consumption”) for use under P-limiting conditions (Solovchenko et al., 2019). Past studies have demonstrated that algae can increase intracellular P storage in response to episodic P additions (Humphrey and Stevenson, 1992; Rier et al., 2016) and microscale P pulses have been shown to influence the community structure and function of algal biofilms (Davies and Bothwell, 2012). Moreover, the potential for algal communities to utilize short duration increases in P has been demonstrated through increased P retention in stream nutrient addition experiments (Meals et al., 1999; Weigelhofer et al., 2018). However, past investigations on the effects of P pulses have occurred in conditions with low total P loads or have concentrated on stream biogeochemistry, thus the potential of episodic P enrichment to augment stream eutrophication remains unclear. Understanding the capacity of benthic algal communities to respond to contrasting temporal patterns of P enrichment associated with point and non-point source P loading is thus required to inform stream eutrophication monitoring and management in developed watersheds.

The goal of our study was to determine the effect of temporal patterns in P enrichment, consistent with point source and non-point source inputs, on the growth and taxonomic composition of benthic algal communities. To accomplish this goal we conducted a mesocosm experiment in artificial streams where algal communities were exposed to P loadings that simulated unenriched, continuously enriched, or episodically enriched conditions, with P loads over the duration of the experiment being the same for the two P enriched treatments. Mesocosm experiments provide the opportunity to examine more nuanced effects of P enrichment by balancing ecological realism with added control in environmental conditions and replicability

(Menczelesz et al., 2019). Thus, our experimental research can provide unambiguous evidence on the potential for algal communities to respond contrasting patterns of P enrichment and help inform future field studies. Findings from our experiment will also directly inform stream eutrophication management and the refinement of ecological indicators for monitoring in streams draining mixed land use catchments.

2. Methods

2.1 Experimental Design and Set-Up

We used a single factor research design to investigate the response of algal communities to simulated P enrichment patterns associated with three different land use scenarios in a 29-day artificial stream mesocosm experiment. Nine artificial streams were randomly assigned to one of three different P treatments: 1) an unenriched P load with a continuous concentration of 10 µg L^{-1} SRP (unenriched); 2) an enriched P load with a continuous P concentration of 50 µg L^{-1} SRP (continuously enriched), and; 3) an enriched P load with a continuous concentration of 10 µg L^{-1} SRP that received two separate 48-hour additions (“pulses”) of P at 300 µg L^{-1} SRP on days 10 and 19 (episodically enriched). P enriched treatments were selected such that total P loads at the end of the 29-day experiment were the same for continuously and episodically enriched treatments (Fig. 1). Total nitrogen was constant for all treatments at a continuous concentration of 1500 µg L^{-1}. Experimental P concentrations were chosen with regard to regional nutrient criteria (Chambers et al., 2012) and reflected concentrations observed in human influenced streams of southern Ontario, Canada (Thomas et al., 2018).

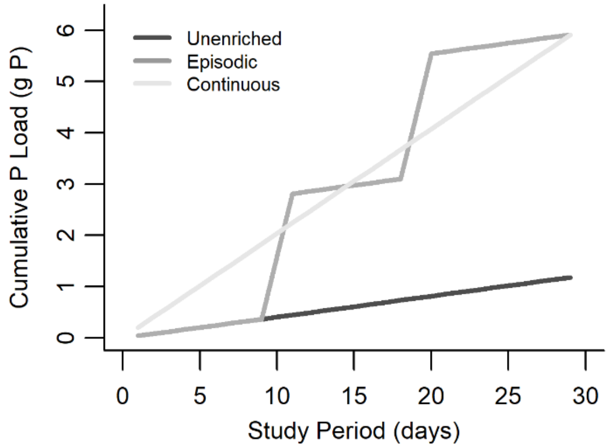

Fig. 1. Cumulative experimental phosphorus load for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) over the 29-day experiment.

Our artificial stream mesocosm experiment was conducted at the Thames River Experimental Stream Sciences (TRESS) center in London, Ontario, Canada, in the early summer (June 19 – July 18) of 2016 (Fig. 2). Artificial streams were comprised of sinuous flumes (0.15 m deep by 0.20 m wide by 7.0 m long) that received a continuous supply of low nutrient (TN = 406 µg L^{-1}; TP < 1.0 µg L^{-1}) carbon filtered water from the Lake Huron Water Supply System through individual diaphragm pumps. Concentrated P (KH_2PO_4) stored in 1000 L carboys was delivered to each artificial stream through individual dosing pumps connected via chemical injection fittings to the outflow of each diaphragm pump. Similarly, a single dosing pump was used to add concentrated nitrogen (NH_4NO_3) to the common water supply of the facility. Flow rates of dosing and diaphragm pumps were calibrated daily to ensure concentrated nutrients were continuously delivered at the appropriate rates to achieve and maintain target artificial stream nutrient concentrations. Water and nutrients delivered to each artificial stream were circulated throughout the flume with an impeller pump and were partially recycled with the use of a reservoir (2.5 h residence time). Because of the continuous and controlled delivery of water and nutrients, concentrations in the artificial streams remained temporally stable and were largely calculated based on the amount of P added to the nutrient carboys and the calibrated flow rates of each dosing and diaphragm pump. Water samples were collected on four occasions to confirm treatment P concentrations and were found to be within an acceptable margin of error of calculated concentrations.

Light availability (60% shade) and water temperature were controlled and equal among artificial streams and reflected diurnal cycles and day-to-day variability in ambient climatic conditions (Supplementary Material: Fig. S1). Mean (± standard deviation) daily photosynthetically active radiation and temperature over the experimental period were 27.4 ± 6.2 mol m^{-2} day^{-1} and 20 ± 1.8 ^{o}C, respectively. Flow velocity was set at 0.1 m s^{-1} in all artificial streams and was measured daily to ensure the selected velocity was maintained for the duration of the experiment. Substrate in all artificial streams consisted of cobble (D50 = 46 mm).

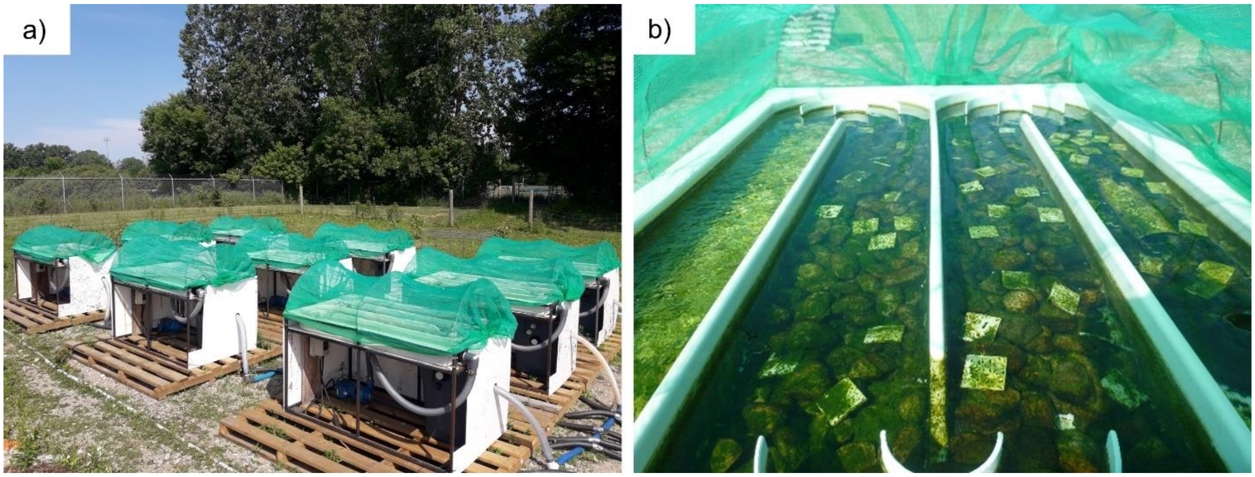

Fig. 2. (a) Nine artificial streams located at the Thames River Experimental Stream Sciences Center in London Ontario, Canada. (b) Close up image of the artificial stream flume and substrate part way through the experiment.

Prior to the experiment, cobble substrate was placed in plastic mesh (2 x 2 cm) bags and anchored in a local stream (43.013, -81.281) for approximately 4 weeks to colonize artificial stream substrate with local biota. Colonized substrate was divided equally among each artificial stream at the beginning of the experiment to achieve a water depth of ~ 8 cm. Additional algae inoculum was collected from 5 regional streams that occupied a gradient of P enrichment (19.6 µg L^{-1} TP to 229.5 µg L^{-1} TP) by removing the biofilm from 5 to 10 cobbles randomly selected within each reach. Collected biofilms were combined in 2.7 L of water and used to seed each artificial stream (300 mL) with biota representative of the regional P conditions. Unglazed ceramic tiles (4.7 x 4.7 cm) were placed on top of the pre-colonized substrate to provide a clean surface for biofilm growth. Tiles and substrate were redistributed randomly within each treatment group 5 days into the experiment to further establish homogeneity among artificial streams. Large grazers (e.g., snails and crayfish) were removed from artificial streams at the beginning of the experiment and because substrate was inoculated in the same stream and randomly distributed among artificial streams grazing by small invertebrates was presumed to be consistent among streams.

2.2 Sample Collection and Analysis

Standardized substrata (unglazed ceramic tiles; 22.09 cm^2) were placed in each artificial stream at the start of the experiment to provide a consistent surface for benthic algal colonization and accrual (sensu Steinman et al., 2007). Algal samples were collected on seven occasions during the experiment: prior to the first simulated P pulse (days 7 and 10), between simulated P pulses (days 15 and 18), and after the second simulated P pulse (days 22, 25, and 29). Samples were collected for chlorophyll-*a*, ash-free dry-mass (AFDM), and taxonomic identification (algal phylum and diatom species). For each measurement, a composite sample was collected by scraping a defined surface area of biofilm (5.31 cm^2) from four different tiles (21.24 cm^2). Sampled tiles were selected at random with at least one tile being selected from each of the three flume channels (Fig. 2). Each selected tile was randomly split into two diagonal sections and a tubular sampler (Steinman et al., 2007) was used to collect one scraping of biofilm (5.31 cm^2) from each section for biomass (either chlorophyll-*a* or AFDM) and taxonomy. Composite samples were collected to account for potential spatial heterogeneity in algal accumulation within individual artificial streams and a tubular sampler was used to increase the consistency in and number of samples collected. Sampled tiles were removed from the experiment.

Chlorophyll-*a* samples were stored frozen and thawed prior to fluorometric analysis. Thawed samples were filtered through Whatman GF/C filters and placed in 50 mL centrifuge tubes with 10 mL of 90% ethanol. A hot ethanol non-acidification extraction was completed by partially submerging centrifuge tubes in an 80 ^oC hot water bath for 7 min. Liquid extract was diluted if necessary and chlorophyll-*a* concentration was determined with a Turner Designs Trilogy Fluorometer (Model: 7200-000).

Ash-free dry-mass samples were stored frozen before analysis. Thawed samples were filtered onto pre-ashed Whatman GF/C filter papers for organic mass determination. Filtered samples were dried at 105 ^oC for a minimum of 12 hours and weighed. Samples were then ashed in a muffle furnace at 550 ^oC for 1 hour and subsequently weighed to determine the mass loss on ignition.

Taxonomy samples were preserved in dark bottles with Lugols iodine (~ 1% v/v) and subsampled for taxonomic identification. Coarse algal taxonomy was determined by sub-sampling 0.35 mL of well-mixed sample into a 4.25 mL Utermöhl chamber and diluting with 3.9 mL of distilled water. Algal cells were allowed to settle for 3 to 4 hours and were then enumerated (minimum 400 cells) by coarse taxonomic level (i.e., phylum) with the use of an inverted microscope at 400x magnification.

Diatom taxonomy was determined by digesting biofilm subsamples in 800 µL of 100% (v/v) nitric acid for 48 hours and 200 µL of hydrogen peroxide 30% (v/v) for an additional 48 hours to remove organic matter and clean diatom frustules. Digested samples were rinsed several times to remove any remnant nitric acid before being mounted with Naphrax® on microscope slides (refractive index: 1.74; Brunel microscopes Ltd.,Wiltshire, UK). Diatom assemblages were enumerated with use of a Reichert-Jung Polyvar microscope equipped with differential interference contrast (magnification 1250x). A minimum of 400 diatom valves were enumerated for each sample and each valve was identified to lowest possible taxonomic level, usually species, following Lavoie et al. (2008b).

2.3 Data Analysis

Biomass data from the end of the experiment were used to evaluate differences between P treatments because of the temporal discontinuities in the cumulative experimental P load. Algal biomass measures (chlorophyll-*a* and AFDM) were compared with a one-way analysis of variance and a Tukey's post hoc test was used to evaluate pairwise differences among P treatments ($\alpha = 0.05$). Statistical analyses were performed in R version 3.5 (R Core Team, 2019).

Biomass measures were also used in generalized additive models (GAM) to produce nonlinear algal accrual curves and infer absolute growth rates over the experiment by estimating the rate of change in biomass across the seven sampling events (sensu Paine et al., 2012). GAMs were developed with the *mgcv* package (Wood 2019) for each P treatment and described algal accrual as the relationship between biomass and time ($\alpha = 0.05$). Thin plate regression splines were used to smooth the data with a conservative estimated number of degrees of freedom (edf < 7). For all P treatments, model weight was added to samples collected before the first P pulse (days 7 and 10) in the GAM to prevent underfitting during algal colonization. Absolute growth rates were estimated as the first derivatives of each GAM of algal accrual with the *gratia* package (Simpson, 2019).

Composition of diatom assemblages before (day 10) and after (day 25) simulated P pulses were compared among P treatments using a non-metric multidimensional scaling (nMDS) ordination. Assemblages were ordered based on a Bray-Curtis dissimilarity matrix of square root transformed species relative abundance data (Legendre and Gallagher, 2001). A permutational analysis of variance was performed to evaluate the factors of P treatment, time (before and after), and their interaction ($\alpha = 0.05$). Confidence intervals (95%) based on standard deviations of each P-time treatment level were computed to evaluate pairwise differences, and a similarity percentages (SIMPER) analysis was used to determine the diatom species responsible for dissimilarity among treatments. Analyses were completed with the *vegan* package (Oksanen et al., 2019).

3. Results

3.1 Biomass

Algal biomass measures differed among P treatments at the end of the experiment for chlorophyll-*a* and AFDM (Fig. 3). P enriched treatments were 2-fold greater in chlorophyll-*a* and AFDM compared to the unenriched treatment, but no differences were observed between continuously and episodically enriched treatments that received the same cumulative P load (chlorophyll-*a*: $F_{(2, 6)} = 16.35$, $p = 0.004$; AFDM: $F_{(2, 6)} = 11.31$, $p = 0.009$).

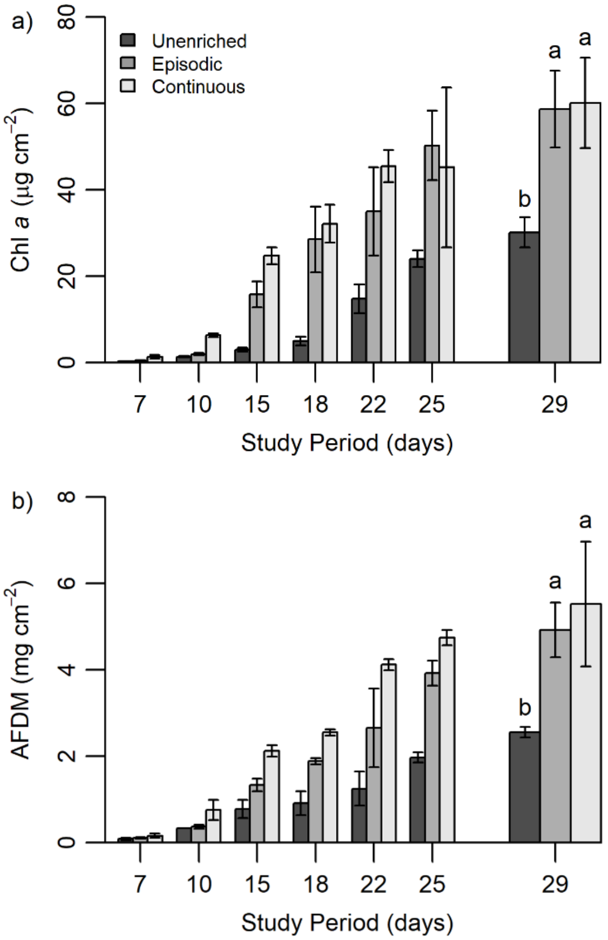

Fig. 3. Algal biomass (mean ± 95% confidence interval) for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) measured as (a) chlorophyll-*a* (Chl *a*) and (b) ash-free dry-mass (AFDM) from seven sampling events over the 29-day experiment. Letters (a, b, and c) indicate significant ($p < 0.05$) pairwise differences based on individual Tukey's post hoc test for the final sampling event.

3.2 Algal Accrual

GAMs of algal accrual indicated that chlorophyll-*a* and AFDM from each P treatment were associated with time (Fig. 4). GAMs explained 99.0%, 97.2%, and 96.0% of the deviance in chlorophyll-*a* accrual curves for unenriched (edf = 5.64, $p < 0.001$), episodically enriched (edf = 3.72, $p < 0.001$), and continuously enriched (edf = 3.56, $p < 0.001$) treatments, respectively. Likewise, deviance explained by GAMs of AFDM were 98.3% for the unenriched (edf = 5.28, p

< 0.001), 98.0% for the episodically enriched (edf = 3.14, $p < 0.001$), and 97.0% for the continuously enriched (edf = 3.44, $p < 0.001$) treatments.

Absolute growth rates inferred from the first derivative of chlorophyll-*a* accrual curves were similar between unenriched (0.29 µg cm^{-2} day^{-1}) and episodically enriched (0.32 µg cm^{-2} day^{-1}) treatments before simulated P pulses (day 7), whereas the continuously enriched treatment had a greater initial absolute growth rate of chlorophyll-*a* (1.22 µg cm^{-2} day^{-1}) (Fig. 4). Following the first P pulse (day 13), the absolute growth rate in the episodically enriched treatment (2.79 µg cm^{-2} day^{-1}) diverged from the unenriched treatment (0.27 µg cm^{-2} day^{-1}) and experienced more rapid rates of accrual. Absolute growth rates plateaued at a comparable rate of change for both continuously (day 15: 3.32 µg cm^{-2} day^{-1}) and episodically (day 17: 3.35 µg cm^{-2} day^{-1}) enriched treatments, but the episodically enriched treatment required an additional 2 days to reach a maximum. Absolute growth rates in the episodically enriched treatment remained above 3.0 µg cm^{-2} day^{-1} after the second P pulse (day 25), whereas the rate of change in chlorophyll-*a* declined in the continuously enriched treatment to below 2.5 µg cm^{-2} day^{-1}. In contrast, the unenriched treatment exhibited a longer phase of lower absolute growth rates during the first half of the experiment and experienced a peak absolute growth rate (3.09 µg cm^{-2} day^{-1}) approximately 7 days later than the P enriched treatments.

Absolute growth rates inferred from AFDM accrual curves differed from chlorophyll-*a* in that more time was required to reach maximum rates (Fig 4). Initially (day 7), the continuously enriched treatment (0.14 mg cm^{-2} day^{-1}) had a greater absolute growth rate than both episodically enriched (0.07 mg cm^{-2} day^{-1}) and unenriched (0.06 mg cm^{-2} day^{-1}) treatments. After the first P pulse (day 13) absolute growth rates in the episodically enriched treatment (0.17 mg cm^{-2} day^{-1}) increased and were 2-fold greater than the unenriched treatment (0.09 mg cm^{-2}

day^{-1}), yet accrual remained lower in comparison to the continuously enriched treatment (0.25 mg cm^{-2} day^{-1}). Following the second P pulse (day 25) absolute growth rates in the episodically enriched treatment remained above 0.29 mg cm^{-2} day^{-1} whereas absolute growth rates in the continuously enriched treatment declined to less than 0.25 mg cm^{-2} day^{-1}. However, maximum absolute growth rates were comparable between continuously (0.28 mg cm^{-2} day^{-1}) and episodically (0.29 mg cm^{-2} day^{-1}) enriched treatments, and greater than the unenriched treatment (0.19 mg cm^{-2} day^{-1}). Maximum absolute growth rates occurred on days 19, 24, and 29 for continuously enriched, unenriched, and episodically enriched treatments, respectively.

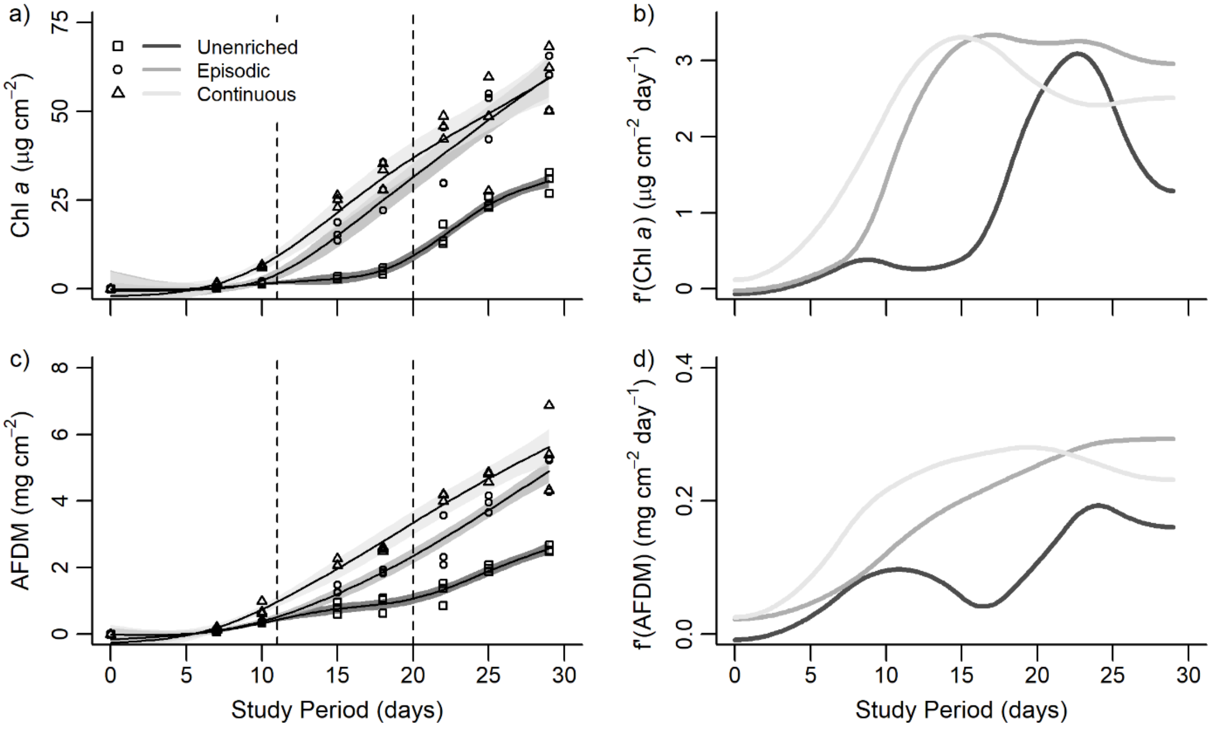

Fig. 4. Generalized additive models (left) of algal biomass accrual over the 29-day experiment for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) measured as (a) chlorophyll-*a* (Chl *a*) and (c) ash-free dry-mass (AFDM). First derivatives of generalized additive models (right) correspond to the absolute growth rates of algal biomass measured as (b) chlorophyll-*a* (Chl *a*) and (d) ash-free dry-mass (AFDM). Dashed lines represent timing of simulated phosphorus pulses.

3.3 Taxonomic Composition

Relative abundances of algal phyla were comparable between unenriched and episodically enriched treatments prior to simulated P pulses; however, benthic algal composition of both these treatments differed from that of the continuously enriched treatment (Fig. 5). On day 10 of the experiment, communities that received continuous enrichment had 30% more chlorophytes and 25% fewer bacillariophytes compared to communities from unenriched and episodically enriched treatments. Following simulated P pulses, relative abundances of algal phyla in the episodically enriched treatment shifted and were comparable to the continuously enriched treatment with a 60% relative abundance of chlorophytes in both treatments (Fig. 5). Proportions of chlorophytes in the unenriched treatment remained at 30% over the experiment, but the relative abundance of charophytes (e.g., *Cosmarium sp.*) increased by 20% with a 25% reduction in bacillariophytes towards the end of the experiment. Chlorophyta was the dominant phylum responsible for differences observed in P enriched and unenriched treatments after simulated P pulses.

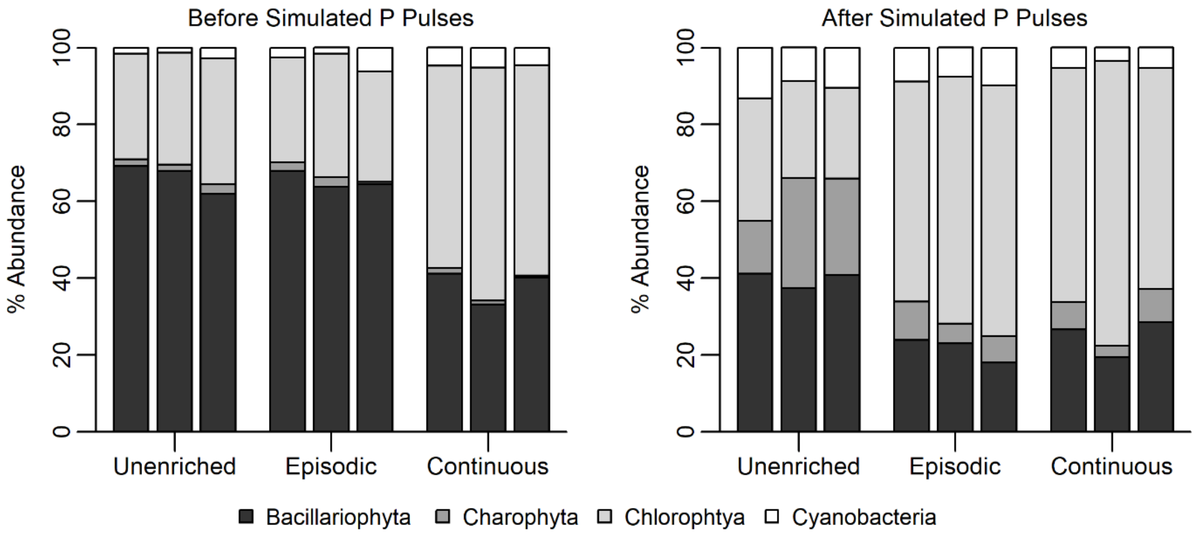

Fig. 5. Relative abundances of algal phyla (dark grey: Bacillariophya, medium grey: Charophyta, light grey: Chlorophyta, white: Cyanobacteria) in communities of the three phosphorus

treatments (three replicates per treatment) sampled before (left) and after (right) simulated phosphorus (P) pulses.

An nMDS ordination (stress = 0.06) analyzing a total of 28 diatom species observed in our artificial streams showed the separation of assemblages in two dimensions (Fig. 6). On average, 21, 20, and 20 diatom species were recorded before and 12, 14, and 17 after simulated P pulses for the unenriched, episodically enriched, and continuously enriched assemblages, respectively. Assemblages were clustered by P treatment ($F_{(2, 17)} = 6.24$, $p < 0.001$, $R^2 = 0.21$) and time ($F_{(1, 17)} = 28.44$, $p < 0.001$, $R^2 = 0.47$), which cumulatively explained 68% of the ordination variance based on a permutational analysis of variance. A total of 81% of the variance in diatom assemblages was explained with the addition of a significant P-time interaction ($F_{(2, 17,)} = 3.83$, $p = 0.004$, $R^2 = 0.13$). For samples collected before simulated P pulses 95% confidence intervals overlapped for all P treatments. However, after simulated P pulses each treatment occupied separate ordination space. Continuously and episodically enriched diatom assemblages were positively scaled on nMDS axis 2 and were more dissimilar to the negatively scaled unenriched assemblages than to each other.

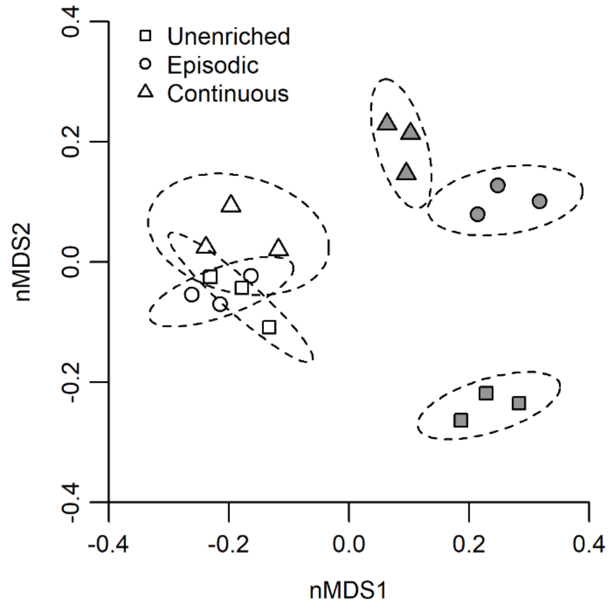

Fig. 6. Non-metric multidimensional scaling ordination of diatom species composition of phosphorus treatments (square: unenriched, circle: episodic, triangle: continuous) collected before (open symbols) and after (closed symbols) simulated phosphorus pulses. Dashed lines represent 95% confidence intervals based on standard deviations.

SIMPER analysis identified that after simulated P pulses diatom assemblage composition of the unenriched treatment was about 40% dissimilar to continuously and episodically enriched treatments, whereas P enriched assemblages were 28% dissimilar (Table 1). Four influential diatom taxa were each responsible for greater than 10% of the relative dissimilarity observed among treatments. *Nitzschia palea*, *Nitzchia palea* var. *debilis*, and *Achnanthidium minutissimum* contributed most to the dissimilarity between the P enriched and unenriched diatom assemblages, whereas *Cyclotella meneghiniana* and *A. minutissimum* contributed to the dissimilarity between episodically and continuously enriched diatom assemblages.

Table 1. Similarity percentages analysis of the average dissimilarly (Av. Dissim.) in diatom assemblage composition among phosphorus treatments after simulated phosphorus pulses. Influential diatom taxa were described based on the absolute contribution (Abs. Contrib.) to the

total dissimilarity between treatments and identified as important based on a >10% relative contribution (Rel. Contrib.).

Treatment Comparison	Av. Dissim.	Influential Taxon	Abs. Contrib.	Rel. Contrib.
Unenriched vs. Continuous	44.8%	*Achnanthidium minutissimum*	7.9%	17.6%
		Nitzschia palea	7.8%	17.4%
		Nitzschia palea var. *debilis*	5.1%	11.4%
Unenriched vs. Episodic	36.4%	*Nitzschia palea*	6.3%	17.3%
		Achnanthidium minutissimum	4.7%	12.9%
		Nitzschia palea var. *debilis*	4.0%	11.0%
Continuous vs. Episodic	28.0%	*Cyclotella meneghiniana*	3.9%	13.9%
		Achnanthidium minutissimum	3.1%	11.1%

Relative abundances of influential diatom taxa were comparable among P treatments before simulated P pulses, but differed with the succession of algal communities (Fig. 7). Relative abundance of *A. minutissimum* increased over the experiment in the unenriched treatment and was 2 to 3-fold more abundant than in the continuously and episodically enriched treatments after simulated P pulses. In contrast, *N. palea* and *N. palea* var. *debilis* increased in continuously and episodically enriched treatments such that after simulated P pulses both taxa had relative abundances 30 to 40% greater than the unenriched treatment. Continuously and episodically enriched treatments differed by 20%, 5%, and 10% in the relative abundances of *A. minutissimum*, *N. palea* and *N. palea* var. *debilis*, respectively, with the latter two species being more abundant in the continuously enriched treatment and the former in the episodically enriched treatment. Moreover, after simulated P pulses *C. meneghiniana* was only found to be abundant in the continuously enriched treatment (8%) in comparison to episodically enriched and unenriched treatments (< 1%).

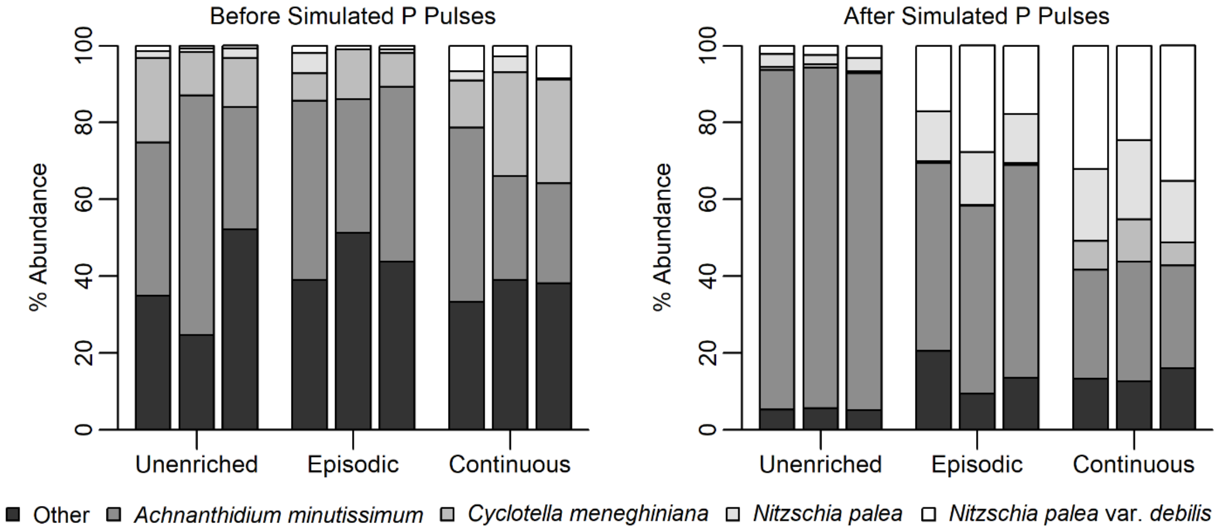

Fig. 7. Relative abundances of influential diatom species identified through similarity percentages analysis (dark grey: Other, medium-dark grey: *Achnanthidium minutissimum*, medium grey: *Cyclotella meneghiniana*, light grey: *Nitzschia palea*, white: *Nitzschia palea var. debilis*) in assemblages of the three phosphorus treatments (three replicates per treatment) sampled before (left) and after (right) simulated phosphorus (P) pulses.

4. Discussion

Ecological theory suggests that resource availability regulates community composition (habitat template model; Biggs et al., 1998). Although numerous studies have investigated how benthic algal communities are structured by instream P availability (Biggs, 2000; Chételat et al., 1999; Stevenson et al., 2008; Stevenson et al., 2012), most research has focused on ecological responses wherein the supply of P has differed spatially. Few studies have examined how algal communities respond to temporally variable P supplies associated with human activities (Humphrey and Stevenson, 1992; Rier et al., 2016). Moreover, there have been few comparisons of the eutrophication potential of P enrichment from sources differing in temporal loading pattern. By simulating continuous and episodic P enrichment patterns in artificial streams, we found that the total P load regulated algal biomass and composition of algal phyla, whereas the combination of P load and temporal loading pattern structured community growth rate and diatom species composition. Our findings suggest that episodic P pulses can have significant effects on the structure of algal communities and thus runoff driven P loading may play a largely unacknowledged role in structuring benthic algal communities in streams.

4.1 Algal Biomass

We observed that biomass of benthic algal communities increased with P enrichment and nearly doubled with a 5-fold increase in total P load. Numerous studies have observed such increases in benthic algal biomass with increasing P supply (Biggs, 2000; Chételat et al., 1999; Stevenson et al., 2012). However, contrary to the presumption that algal P utilization is limited by the short residence time of episodic inputs we also observed that peak biomass of algal communities did not differ whether P was supplied continuously or in the form of two P pulses that, together, delivered 83% of the cumulative experimental total P load. Thus, our findings support the small number of studies that have shown structural and physiological responses of algal communities to short duration increases in P (Humphrey and Stevenson 1992; Rier et al., 2016). Moreover, our study builds on past research by revealing that the total P load a community receives may be more important in the regulation of algal biomass than P delivery pattern. Davies and Bothwell (2012) made a similar observation in a stream mesocosm experiment that simulated microscale temporal variation in P (e.g., invertebrate grazing and excretion) whereby the hourly integrated P load controlled algal biomass opposed to the duration of sub-hourly P pulses (1–60 min h^{-1}) differing in concentration. Although we observed the same regulation of benthic algal biomass by total P load as Davies and Bothwell (2012), the simulation of P enrichment patterns associated with human land use permits our findings to be directly translatable to P enrichment in streams that drain developed watersheds.

Our finding that episodic P pulses have the potential to stimulate and sustain the development of algal mats demonstrates the importance of short-duration P subsidies on algal biomass accrual. Short-term algal accrual curves differed among P treatments such that the onset of exponential growth and maximum absolute growth rates occurred earlier in the P enriched treatments than the unenriched treatment. Observed effects of P enrichment on absolute growth rates is consistent with past studies that have demonstrated that resource availability can stimulate the rate of change in algal biomass (Rier et al., 2006; Fellows et al., 2006) and govern temporal patterns in algal accrual (Bothwell, 1989).

We also found that although the maximum absolute growth rates attained were comparable between P enriched treatments, the onset of exponential growth and maximum absolute growth rates occurred later in the episodically enriched communities and was associated with the first P pulse. Moreover, we observed that absolute growth rates declined towards the end of the experiment in the continuously enriched treatment, but not in the episodically enriched treatment. Algal accrual often declines with community succession as peak biomass is maintained through a period of turnover and low absolute growth rates prior to biomass loss or autogenic sloughing (Biggs, 1996). Our observation of increased absolute growth rates for the episodically enriched treatment indicates the second P pulse prolonged successional transition from biomass accrual to turnover resulting in both P enriched treatments accruing the same amount of biomass at the end of the experiment. Although we did not carry out our experiment long enough to observe autogenic sloughing, biomass differed less than 1.6-fold between P enriched communities over the latter half of the experimental period, suggesting that biomass-mediated effects on resource stress in the basal layers of algal mats (e.g., light attenuation and nutrient diffusion) would have been similar (Hill and Boston, 1991). However, future studies are needed to investigate the long-term temporal patterns in benthic algal succession associated with differences in continuous and episodic P enrichment.

4.2 Algal Community Composition

Relative abundances of algal phyla responded to total P load, but not P enrichment pattern. Diatoms were most abundant at the beginning of our experiment, but P enrichment shifted the composition of algal communities towards a chlorophyte dominated community. Chlorophyte accrual is expected in P-rich environments with ample light and minimal disturbance (Biggs et al., 1998) and algal communities amended with P in other mesocosm experiments have shown similar shifts in chlorophyte relative abundance (Bækkelie et al., 2017; Bondar-Kunze et al., 2016). Moreover, filamentous chlorophyte abundance is often associated with the increased trophic status of streams (Chételat et al., 1999; Stevenson et al., 2012). Although the largest fraction of chlorophyte taxa enumerated in our study were non-filamentous (*Scenedesmus* sp. and *Pediastrum* sp.), our study also observed an increase in filamentous chlorophytes and as a group chlorophytes appeared to be the cause of eutrophic or nuisance levels (sensu Suplee et al., 2009; Welch et al., 1988) of algal biomass in the P enriched communities. Thus, P enrichment from continuous and episodic sources may result in a comparable aesthetic of benthic algal communities and similar loss of valued ecological conditions.

We observed that diatom assemblage composition was initially comparable among P treatments, but diverged with enriched P loads. Our finding that P enriched diatom assemblages were dissimilar to unenriched assemblages is consistent with past studies that have investigated the response of diatoms to stream P gradients (Black et al., 2011; Taylor et al., 2018) and human land use patterns (Walsh and Wepener, 2006). However, we also found differences in diatom assemblage composition associated with the temporal pattern of P loading as continuously and episodically enriched assemblages were significantly dissimilar. The differences we observed between P enriched diatom assemblages supports past research linking temporal variability in diatom assemblage composition with changes in P availability in individual streams (e.g., Lavoie et al., 2008a; Snell et al., 2014). Thus, distinct diatom assemblages observed among P treatments indicates that both amount and pattern of P enrichment may regulate species performance and manifest in assemblage-level differences in diatom composition.

Four diatom species contributed most to the observed dissimilarity in diatom assemblage composition among P treatments. *Nitzchia palea* and *Nitzchia palea* var. *debilis* had greater relative abundances in P enriched assemblages, whereas *Achanthidium minutissimum* had a greater relative abundance in unenriched assemblages. *N. palea* has an optimal total P concentration 2-fold greater than *A. minutissimum* (Ponader et al., 2007), which coincides with the aforementioned differences observed in relative abundance. However, *N. palea* did not contribute to the dissimilarity between continuously and episodically enriched assemblages. Comparable relative abundances of *N. palea* in P enriched assemblages indicates that *N. palea* can effectively capture resources from episodic increases in P while also benefiting from continuously enriched conditions. In contrast, *Cyclotella meneghiniana* was largely responsible for the dissimilarity between continuously and episodically enriched assemblages. Yet, although *C. meneghiniana* has a total P optimum comparable to *N. palea* (Ponader et al., 2007), *C. meneghiniana* may have limited success in P limited environments through a passive cellular morphology (Grover 1989) and low potential for P storage (Kilham et al., 1977; Tilman and Kilham 1976). Indeed, greater relative abundances of *C. meneghiniana* observed in continuously enriched assemblages further suggests that this species may be unable to effectively capture resources in streams with high temporal variation in P concentrations. P optima of benthic diatoms may therefore not fully represent the most favourable conditions for success as the temporal availability of P appears to have a detectable effect on at least one species observed in our study.

Streams that served as the species pool for our experiment frequently receive increased loads of P from point and non-point sources within their catchments. Algae communities in artificial streams at the start of our experiment were thus representative of taxa commonly found in mesotrophic to eutrophic streams located in a temperate region where P enrichment is an important management concern. Therefore, the compositional differences we observed should be representative of the differential effects that continuous and episodic P enrichment have on instream benthic algae communities. As such our findings are relevant to the management of streams that drain anthropogenic landscapes with P conditions similar to the streams from which our inocula were collected. However, it is unlikely that our starting communities would fully represent those found in oligotrophic streams with catchments exposed to more limited human influence. Consequently, our findings may be less informative for the management of oligotrophic streams as the effects of P loading pattern on algal community composition in our study may have been constrained by the initial species pool used to inoculate artificial streams. For example, Taylor et al., (2018) found that benthic algae communities transferred into stream mesocosms from nutrient poor streams exhibited a larger change in diatom assemblage composition in response to differences in ambient P concentrations compared to communities from more nutrient enriched streams. Future studies are therefore needed to evaluate the compositional response of benthic algal communities found in oligotrophic streams to episodic P enrichment in order to provide further insight for the management of streams where P loadings from human activities are more limited.

4.3 Ecological Potential of P Pulses

Episodic P loading from non-point sources are often accompanied by additional physicochemical changes in stream conditions. Our findings demonstrate the potential of algal communities to accrue biomass from P pluses, but the observed response may not be attainable with extraneous (e.g., season and baseflow P) and co-occurring (e.g., discharge and turbidity) controls on algal development in streams. Specifically, hydrodynamic effects from runoff events can scour benthic environments and lessen the structural response of algae to P inputs (Biggs and Close, 1989). However, disturbance to algal biomass can vary spatially within a reach and temporally with the successional stage of algal communities (Katz et al., 2018; Peterson and Stevenson 1992). Likewise, algae are resistant to small and moderate flow events (Biggs and Close, 1989; Biggs et al., 1998) that can contribute a substantial proportion (37–52%) of the total P load in headwater streams (Macrae et al., 2007). Moderate increases in discharge from sub-scouring events can also promote the diffusion of P into algal communities, thereby increasing P uptake and production (Horner and Welch, 1981; Townsend et al., 2012). P loading from sub-scouring runoff events may therefore have a considerable effect on structuring benthic algal communities in streams (Stevenson, 1990). Moreover, a community-level response to episodic P loadings may be further regulated by background P concentrations (Cook et al., 2018). For example, many experimental studies in more nutrient enriched systems have shown no change in algal accrual or diatom assemblage composition associated with increased P concentrations (Bowes et al., 2012; McCall et al., 2017; Taylor et al., 2018). Algal communities in streams that have enriched background P concentrations may be nutrient saturated and unable to effectively utilize episodic loadings (Weigelhofer et al., 2018). Future studies are therefore needed to incorporate additional realism in order to fully understand the eutrophication potential and ecosystem-level implications of episodic P loadings in streams.

5. Conclusions

5.1 Applications to River Management

The potential for algal communities to respond to episodic P inputs has important implications for watershed management. First, P based monitoring criteria to control algal biomass may be ineffective in streams that meet baseflow P guidelines, but have high temporal variability in P loading. P concentration guidelines often rely on stressor-response curves where spatial patterns of algal biomass are used to establish change points in stream trophic state and level of impairment (Jarvie et al., 2013). However, temporal variation in P may decouple spatial P concentration-biomass relationships and result in the inadequate evaluation of stream ecosystem conditions. For example, prevailing P concentrations of episodically enriched communities in our study (10 $\mu g\ L^{-1}$ SRP) would meet P management guidelines (Evans-White et al., 2013), but measures of algal biomass would be about 4-fold greater than nuisance growth (Suplee et al., 2009; Welch et al., 1988). Moreover, routine water quality monitoring programs often fail to capture short-duration inputs of P and thus may provide incomplete information needed to control eutrophication in episodically enriched streams (Cassidy and Jordan, 2011). We thus recommend that monitoring programs should consider the incorporation of event-flow P monitoring and the adoption of additional ecological indicators that can respond to temporal variation in P (e.g., diatom assemblage composition) to provide managers with a more comprehensive assessment of stream ecosystem conditions.

Second, varied success of diatom species in the acquisition of P from episodic inputs may have applications in the refinement of ecological indicators used for biological monitoring. Indeed, the results from our study suggest that many diatom species can take advantage of short-duration pulses of phosphorus. However, our study identified one species that was more successful in conditions associated with continuous P enrichment. This information could have applications for the refinement of diatom-based indices and selection of sentinel taxa based on autecological characteristics corresponding to optimal conditions of P acquisition (episodic versus continuous). Although sentinel taxa disproportionally successful under episodic P enrichment were not identified, additional information on P acquisition in diatom-based indices could be valuable in diagnosing the presence or latency of non-point source phosphorus pollution. Future experimental and field studies are thus required to determine the ecological success of other diatom species and the potential for sentinel diatom taxa to serve as indicators of the primary source of P to streams.

Lastly, our observation that total P load outweighed delivery pattern in the control of algal biomass can be used to inform P reduction targets. P management in developed watersheds has often differed based on the surface water type under consideration. For streams, P concentrations during summer baseflow are expected to pose the greatest eutrophication risk, whereas the total P load is most relevant to eutrophication in lakes (Stamm et al., 2013). However, our finding of no difference in peak algal biomass with P loading pattern suggests that episodic inputs of P have an equal potential to augment stream eutrophication as enriched baseflow P concentrations. As such, management interventions that reduce the total P load exported from a watershed would provide dual benefits in the reduction of eutrophication risk in downstream lakes and in the control of nuisance algal production in streams. Thus, future management strategies should consider remedial actions that provide the greatest return on investment in P load reduction.

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