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Effects of physical disturbance on phosphorus uptake in temperate stream biofilms

Keith J. Price[1] and Hunter J. Carrick[1,2*]

[1] Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA 16802, USA

[2] Current address: Department of Biology & Institute for Great Lakes Research, Beaver Island Biological Station, Central Michigan University, Mount Pleasant, MI 48859, USA

* Corresponding author email: hunter.carrick@cmich.edu

Abstract

Microbial biofilm nutrient uptake kinetics can provide insight into assimilative mechanisms that regulate stream primary productivity. While kinetic experiments are often performed, little work has estimated uptake in connection with disturbance (removal) or detailed the effects that common scour events may have on benthic microbes; therefore, the goal of this study was to evaluate physical disturbances on benthic stream biofilms to determine effects on phosphorus (P) uptake rate, physiological capability, and abiotic sorption. Artificial substrata were collected from 2 reaches along a temperate stream; resident biofilms were either removed via abrasion (disturbed) or left intact. A series of short-term radiotracer ($H_3^{33}PO_4$) experiments were then conducted to measure P uptake. *In vivo* autofluorescence was measured as a proxy of algal physiological condition. The experiments showed no difference in P-uptake rates (µgP/µgChl/d) between disturbed ($\bar{x} = 0.77 \pm 0.11$ [SE] µgP/µgChl/d) and intact ($\bar{x} = 0.91 \pm 0.17$ µgP/µgChl/d) biofilms (t = 0.69, p = 0.492, df = 33). Further, microbial physiology was not depressed by physical disturbance. While killed samples yielded significantly lower uptake compared to live biota (F = 17.51, p = 0.001), abiotic sorption still accounted for a moderate fraction (range = 0.021–0.038 µgP/µgChl/d) of total uptake and thus warrants estimation in metabolic studies. Overall, these findings lend credence to numerous experiments that investigate benthic microbial physiologic responses post-disturbance and highlight the importance of uptake following common physical disturbances that occur in turbulent environments. In addition, our work measured several kinetic constants across both disturbance and temporal gradients, and we discuss their significance within a physiological framework.

Key words: abiotic sorption, biofilm, C_{min}, cell viability, disturbance, scouring, P storage, uptake

Introduction

Streams are dynamic features of the landscape, in part because they serve as sediment and nutrient transport avenues (Hall et al. 2002); specifically, small streams (width ≤10 m) represent up to 85% of total stream length in most watersheds, provide a crucial link between terrestrial and aquatic environs, and are key elements in nutrient transformation and downstream transport (Peterson et al. 2001, Sweeney et al. 2004). Benthic stream biofilms are capable of assimilating and effectively retaining nutrients (via deposition and burial) that would otherwise be transported downstream (Dodds 2003), although mineralization can exceed uptake in some systems (O'Brien and Dodds 2008). As such, biofilms are critical in the removal (at least temporarily) of dissolved phosphorus (P) from stream water and are major components in stream self-purification (Sabater et al. 2002, Covich et al. 2004) through their conversion of inorganic nutrients into particulate form. This reduces bio-availability and buffers downstream ecosystems from high soluble reactive nutrient deliveries (Svendsen et al. 1995,

Aldridge et al. 2010). Microbial P-uptake capacity, however, is regulated to some extent by internal P pool concentrations (i.e., polyphosphates; Rai and Sharma 2006). Polyphosphates (poly-P) can form from biotic luxury consumption during periods of high nutrient availability (Powell et al. 2008). Such availability likely differs according to cell position in the complex biofilm matrix (Burkholder et al. 1990).

As a result of this intricate structure, some biofilm components may be more susceptible to physical disturbance and removal (i.e., top layer of biofilm matrix); periodic sloughing and storm spates can act to remove portions of the biofilm and transport them downstream (Peterson and Stevenson 1990, Biggs 1996). While physical disturbance and removal of the biofilm is a natural occurrence and previous physiological assays have employed physical abrasion or disturbance as a biofilm extraction method (e.g., Tank and Webster 1998, Thompson and Sinsabaugh 2000, Miranda et al. 2007), the effect that such disruption has on cell viability and nutrient assimilation is largely unknown. Additionally, in uptake experiments, some researchers have inferred rates from direct incorporation of radiolabel into microbes (e.g., Hwang et al. 1998, Scinto and Reddy 2003) while others have used the loss of activity from the overlying water (e.g., Odum et al. 1958, Steinman and Mulholland 2006), the selection of technique depending on the microbial group analyzed (e.g., planktonic vs. benthic). Both techniques are applicable to examining the effects of disturbance on biofilms relative to uptake, however, owing to the more planktonic form assumed by biofilms subsequent abrasion.

Nutrient retention in aquatic systems is hence a consequence of this active biological uptake but also abiotic sorption (Haggard et al. 1999), which can be a considerable, if not the dominant, process under some conditions (Rejmánková and Komárková 2000, Aldridge et al. 2010). Few studies routinely test for abiotic sorption (e.g., Steinman and Mulholland 2006), however, or provide measured abiotic sorption fractions in uptake experiments, and quantitative reports on abiotic sorption rates are varied. For example, Klotz (1985) found that the contribution to P cycling by abiotic processes was much greater in comparison to biotic processes; however, Mulholland et al. (1983) and Scinto and Reddy (2003) found that abiotic process accounted for a much smaller proportion of P uptake (10.3 and <15%, respectively).

Michaelis-Menten (M-M) kinetics have been used to describe nutrient uptake into biofilms (Reuter et al. 1986), facilitating estimations of the maximum uptake rate (V_{max}) and half-saturation constant (K_m), although there are many instances where this model is not supported (e.g., Tarapchak and Herche 1986). Similarly, nutrient uptake by higher plants typically follows M-M saturation kinetics and is described by the parameters V_{max}, K_m, and C_{min}, the minimum nutrient concentration required for uptake to occur (Nielsen and Schjørring 1983, Akhtar et al. 2007). The C_{min} or "threshold" value has not been widely estimated before in the aquatic phycology literature (Aubriot et al. 2000, Wagner and Falkner 2001) but may be an important factor in uptake models.

The objective of this study was therefore to examine the effect of physical disturbance on P-uptake rates; specifically we sought to estimate (1) uptake rates in physically abraded (disturbed) and intact biofilms through single endpoint techniques (Collos 1983), and (2) M-M kinetic parameters (V_{max}, K_m, and C_{min}) in disturbed and intact biofilms along varying time-courses. In addition, cell viability and poly-P concentration following increasing disturbance treatments and abiotic sorption were also investigated.

Methods

P-uptake rates of intact vs. disturbed biofilms (single time point)

A set of 6 unglazed ceramic tiles (surface area = 8.42 cm²) was secured to cement blocks and established in an upstream (40°46′42.96″N; 77°46′10.56″W) and downstream (40°49′19.92″N; 77°50′12.84″W) reach in a third-order stream (Spring Creek) located in a mixed land-use watershed in Pennsylvania (USA). Site locations are located in close proximity to those examined by Godwin and Carrick (2008); briefly, PO_4^- concentrations were below detection (<0.005) and 0.012 mg/L in the upstream and downstream reach respectively, reflecting the variation in land use and land cover between the sites (see Godwin and Carrick 2008 for further biogeochemical site descriptions).

Unglazed tiles have been used extensively as standardized substrate for biofilm colonization and development to reduce sample variability (Lamberti and Resh 1985). The tiles were incubated in each stream reach for 30 days to allow the development of mature biofilm assemblages (Biggs 1988). During retrieval, the tiles were removed from the cement blocks, placed into 60 mL translucent polypropylene (Nalgene) incubation jars filled with site-specific water, and returned to the laboratory. Three tiles retrieved from each site were abraded with a stiff bristled brush, representing the disturbed treatment, and 3 tiles were left intact, representing the undisturbed treatment. One milliliter of carrier-free $H_3^{33}PO_4$ (PerkinElmer) was injected into each processed incubation jar containing biofilm and 50 mL ambient water (activity of ^{33}P = 10 μCi/mL). For the abraded (disturbed) treatment, 1 mL of

abraded biofilm material with water (slurry) was removed from each replicate incubation jar using a sterile repeat pipette 10 minutes after tracer ($H_3^{33}PO_4$) injection. This slurry was then injected into a 12-place Millipore filter manifold fitted with 0.20 μm Versapor filters and 15 mL glass water collection tubes. Vacuum filters (containing labeled biofilm material >0.20 μm) and filtered water were separately placed into labeled 20 mL scintillation vials filled with 5 mL Ecolume scintillation cocktail (ICN Pharmaceuticals, Costa Mesa, CA, USA) and read for activity using liquid scintillation counting (LSC; model LS 6000 IC; Beckman-Coulter, Fullerton, CA). For the intact (undisturbed) treatment, 1 mL of water (overlying biofilms) was removed from each replicate incubation jar using a sterile pipette, again 10 minutes after tracer injection.

No obvious signs of seston were present in these jars; however, filtration was still performed on removed water using a 5 cc BD Luer-lock syringe with interchangeable filters (Acrodisc 13 mm syringe filter with 0.2 μm Supor membrane; Pall Corp., Ann Arbor, MI, USA). Water and syringe filters were again separately placed into labeled 20 mL scintillation vials filled with Ecolume and read for activity using LSC. In disturbed treatments, uptake was therefore estimated through direct incorporation of tracer into microbes (on filter), while in undisturbed treatments uptake was inferred through tracer loss from overlying water.

These experiments differ from kinetic experiments in that only a single time point was sampled (limiting the ability to estimate kinetic parameters); rather, the goal of these experiments was to validate single time point estimates (e.g., Collos 1983) of P uptake between disturbed and intact benthic biofilms. These experiments were performed in triplicate and conducted on 17 October 2008, 3 June 2009, and 11 June 2009. Chlorophyll *a* (Chl) concentrations were determined following standard fluorometric methods using a Turner 10-AU fluorometer (Carrick et al. 1993) and converted to areal estimates (mg/m^2).

M-M parameters along distinct time-courses and short-term flux estimations

Similar to the single time point estimation experiments, an additional set of 20 unglazed ceramic tiles were secured to cement blocks and established in the upstream (40°46′42.96″N; 77°46′10.56″W) reach as before (Spring Creek). Again, tiles supporting intact biofilms were placed into incubation jars after 30 days of residence in the stream. Once tiles were returned to the laboratory, intact biofilms were physically removed (as above) from 10 of the collected tiles while the other 10 tiles were left with intact biofilms. Next, all 20 experimental containers were amended with increasing concentrations of KH_2PO_4

(0–500 μgP/L). This concentration range was used in a conservative attempt to capture subsaturated through saturated regions of the hyperbolic M-M uptake curve based on Borchardt (1996), who reported half-saturation constants for periphyton between 0.62 and 1271 μgP/L.

Duplicate experimental jars were amended with 5 P concentrations [P]: 0 (control), 10, 20, 200, and 500 μgP/L (n = 10). For the abraded (disturbed) treatments, 1 mL of abraded biofilm material with water (slurry) was removed from each replicate jar using a sterile repeat pipette 5, 12, 18, 30, and 60 minutes after tracer ($H_3^{33}PO_4$) injection. As before, samples were injected into a filter manifold and subsequently placed into labeled 20 mL scintillation vials filled with scintillation cocktail and read for activity using LSC. For the intact (undisturbed) treatments, 1 mL of water (overlying biofilms) was removed from each replicate jar using a sterile pipette, again 5, 12, 18, 30, and 60 minutes after tracer injection. Seston uptake was measured in a previous experiment and determined to be a negligible component; however, filtration was still performed prior to removing water. The radioactivity in these samples was determined again by LSC.

P storage across growth form and cell viability

The effect of increasing levels of disturbance on biofilm growth form recovery, poly-P concentration, and cell physiology was examined by using 3 different physical abrasion treatments with a stiff-bristled brush (3 rocks per treatment): 1 pass (low disturbance), 2 passes (medium disturbance), and multiple passes (high disturbance). Note that these are more qualitative treatments because we did not directly quantify the level of disturbance (see discussion). For identification of soft algae, 20 mL from each disturbance treatment (n = 9) was preserved with 1% formalin, and cells were identified to genus under 100× and 400× magnifications using a Leica light microscope (Carrick and Steinman 2001). Large (≈300 μm) cells were identified to genus first under 100× magnification using a Palmer-Maloney counting chamber (394 mm^2 in area, 0.1 mL volume). Remaining cells were counted at 400× magnification (random fields) to a minimum of 200 cells until 400 cells total were reached under both 100× and 400×. Cells were then grouped by growth form (filamentous, stalked/erect, and prostrate/adnate). Physiognomic (growth form) classifications of microbial genera followed Graham and Vinebrooke (1998), Wellnitz and Ward (2000), and Passy (2007).

Samples from experiments were filtered (Whatman EPM 2000 glass fiber) for poly-P via hot-water extraction and analyzed using spectrophotometry following standard methods (Fitzgerald and Nelson 1966, Eixler et al. 2005). The percent poly-P contained in each growth form's cells

was calculated by multiplying the total mean poly-P in each disturbance treatment by the mean percent growth form in the treatment, assuming all cells equally distributed the total poly-P. These samples were also analyzed for Chl content using a Turner 10-AU fluorometer (Carrick et al. 1993).

Autofluorescence was measured (via fluorometry) as a proxy of algal physiological condition (n = 9, 3 rocks/disturbance treatment). Sample was poured into a cuvette and read *in vivo* on a fluorometer. Cuvettes were then placed in dark conditions for 1 minute and 0.1 mL of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, a photosynthetic inhibitor) stock (10^{-3} M) was added to each sample (final concentration = 10^{-5} M), which blocks electron transport from photosystem II to photosystem I resulting in maximum fluorescence (Prézelin 1981). Cellular fluorescence capacity (CFC; proportion of absorbed light being used in photosynthesis) was calculated as

$$(F_a - F_b)/F_a, \qquad (1)$$

where F_a is *in vivo* fluorescence post DCMU addition, and F_b is *in vivo* fluorescence (Vincent 1980, Vyhnalek et al. 1993). CFC should vary directly with photosynthetic activity (physiological condition) and inversely with negative effects on photosynthetic activity (e.g., physical disturbance; Vincent 1981, Thompson 1997).

Abiotic sorption

The nonbiological (abiotic) sorption of P by stream benthic biofilms was also investigated using natural rock substrates collected from the same upstream and downstream reaches (Spring Creek). On 17 June 2009, biofilms were physically removed from rock substrates in the field, washed into labeled containers, and returned to the laboratory. Slurries from each site (n = 16) were incubated with the following agents: (1) 10% formaldehyde, (2) 3% glutaraldehyde (Wolfstein et al. 2002), and (3) control (no inhibitory agents).

Sixty minutes after agents were injected into incubation jars, 0.50 mL of carrier-free $H_3{}^{33}PO_4$ radiotracer was injected into each sample. After 10 minutes, slurry was removed from each incubation jar and filtered. Filters were thoroughly rinsed of any nonspecific ^{33}P binding, placed into scintillation vials filled with 5 mL Ecolume scintillation cocktail, and activities estimated via LSC. Note that this was a first-order estimation because the effect of formaldehyde or glutaraldehyde fixation on the possible sorption of P is unknown. Further, no measurements were made of metabolism prior to tracer addition, and while the use of formaldehyde and glutaraldehyde to estimate abiotic uptake is well published in the aquatic phycology literature, we have no absolute assurance that biotic uptake was arrested and therefore note that our abiotic adsorption rates may be overestimates.

Statistical analyses

A mixed-model 3-way analysis of variance (ANOVA) was performed to evaluate variation in P-uptake rate (μgP/μgChl/d) between disturbance treatment (abraded and intact biofilms), site location (up and downstream), and experiment time (sampling dates: 17 October 2008, 3 June 2009, and 11 June 2009). Site was treated as a random effect here because there were no a priori predictions regarding differences among particular stream locations; therefore, a mixed-model ANOVA approach was most applicable (McKone and Lively 1993). Uptake rates were calculated as ln $[P_0/(P_0-X)]$ over time, where P_0 is the total activity in 1 mL of radiolabelled sample and X is the radioactivity on the filter (biota), following Hwang et al. (1998). For statistical comparisons, we determined the uptake into the biota from the intact samples by subtracting the activity measured in the water and activity on the filter (seston) from the total activity. ANOVA was also used to analyze the differences between controls and poisoning agents in estimating biotic versus abiotic uptake.

All descriptive statistics and ANOVA analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). M-M parameters (V_{max} and K_m) were estimated using an iterative "nls" function (nonlinear least-squares regression) in the statistical package R (R Development Core Team 2006, Marino et al. 2010). Data were fitted by nonlinear regression according to the M-M equation using GraphPad Prism 5 (GraphPad Prism Software Inc., San Diego, CA, USA). Observations with difference in fit standardized (DFFITS)values $>2\sqrt{(k/n)}$, where k is the number of predictors (including constant), were removed from regression analyses, following Belsley et al. (1980). To estimate C_{min}, or the threshold [P] at which net incorporation of P by the biofilm ceases due to insufficient available energy (Aubriot et al. 2000), a plot of the uptake rate versus the logarithm of the external P concentration (Thellier plot; Thellier 1970) was made; the intercept on the log [P] axis corresponds to the logarithmic P threshold concentration (i.e., C_{min}; Wagner et al. 1995).

Results

In general, no overall difference in biomass specific P-uptake rate (μgP/μgChl/d) estimates was detected between the 2 methods, disturbed versus intact biofilms (t = 0.69, *p* = 0.492, df = 33); area specific P-uptake rates

Table 1. Three-way mixed-model ANOVA testing the main fixed effects of treatment (n = 2) and experiment (n = 3) and random effect of site (n = 2) on P-uptake rates (µgP/µgChl/d) for intact vs. disturbed stream biofilms. Note: DF = degrees of freedom, F = F-test statistic, and p = p-value.

Source	DF	Sequential sums of squares	Adjusted mean square	F	p
Site	1	10.7368	10.6698	42.83	0.683
Treatment	1	0.2018	0.2493	0.36	0.657
Experiment	2	0.9915	0.4958	22.38	0.043
Site * Treatment	1	0.7827	0.6970	1.48	0.347
Site * Experiment	2	0.0443	0.0222	0.05	0.955
Treatment * Experiment	2	1.4666	0.7333	1.55	0.393
Site * Treatment * Experiment	2	0.9476	0.4738	14.49	0.000
Error	28	0.9158	0.0327		
Total	39	16.0873			

(µgP/m^2/d) also showed no difference (t = 0.96, p = 0.342, df = 31), averaging 29 923 ± 30 664 (SD) and 42 435 ± 49 272 for disturbed and intact assemblages, respectively. The disturbance process yielded a relatively lower coefficient of variation ($\bar{x}$ = 18.1%) among P-uptake estimates by the biofilms, while the P uptake by intact biofilms yielded a comparably higher coefficient of variation ($\bar{x}$ = 25.4%).

Because P uptake was measured for both intact and disturbed assemblages across spatial and temporal scales, we evaluated these influences on the resulting rates (Table 1). As above, abrasion was found to have no effect on P-uptakes rates, and thus no treatment effect (intact, disturbed) was observed. There was a significant temporal component to the variation ($F_{2, 28}$ = 22.38, p = 0.043), such that uptake by biofilms during the fall experiment on 17 October 2008 ($\bar{x}$ = 1.03 ± 0.57 [SD] µgP/µgChl/d) was greater compared with uptake by biofilms sampled from both the first ($\bar{x}$ = 0.75 ± 0.77 µgP/µgChl/d) and second ($\bar{x}$ = 0.68 ± 0.58 µgP/µgChl/d) spring experiments on 3 and 11 June 2009, respectively.

Physical disturbance (abrasion) did not seem to negatively affect cellular function as expressed through function of the photosystems; ANOVA results showed no significant differences in CFC values among disturbance intensities ($F_{2, 6}$ = 2.73, p = 0.144). The various physical disturbance treatments segregated organisms within the biofilm (possessing specific internal P storage capacities). Biologically bound P (poly-P) varied significantly among treatments ($F_{2, 6}$ = 11.34, p = 0.009); specifically, the low treatment averaged 0.133 ± 0.028 (SD), the medium averaged 0.051 ± 0.003, and the high averaged 0.080 ± 0.024 mgP/mgChl. The higher poly-P content in the low disturbance treatment was likely linked to the higher presence of filamentous taxa in the upper strata of the biofilm (Fig. 1).

Killed controls showed that abiotic sorption yielded significantly lower uptake compared to biotic uptake ($F_{2, 9}$ = 17.51, p = 0.001); specifically P-uptake rates averaged 0.068 ± 0.017 (SD), 0.029 ± 0.01, and 0.025 ± 0.00 µgP/µgChl/d by control, formaldehyde, and glutaraldehyde treatments, respectively. Average abiotic sorption (glutaraldehyde and formaldehyde) across both sites sampled accounted for approximately 40% of total P uptake relative to control treatments.

The nonlinear (weighted) least-squares analysis of the M-M model for the disturbed assemblages yielded a V_{max} of 2.25 ± 0.77 (SE) µgP/µgChl/d and K_m of 231.36 ± 140.92 µgP/L (r^2 = 0.99); intact assemblages yielded a V_{max} of 2.33 ± 0.63 µgP/µgChl/d and K_m of 293.87 ± 173.95 µgP/L (r^2 = 0.98). A one-way ANOVA of P-uptake rate (µgP/µgChl/d) versus treatment showed that there was no difference (F = 0.72, p = 0.408) in uptake rates between disturbed and intact assemblages over the 60 minute period. We then tested re-estimated uptake constants (k) by splitting samples between short time periods (i.e., 5–12 min) and long time periods (i.e., 30–60 min) to estimate the effect of experiment duration on the uptake constant and M-M parameters.

For short time periods the disturbed assemblages yielded a V_{max} of 16.64 ± 1.40 (SE) µgP/µgChl/d and K_m of 1457.10 ± 139.35 µgP/L (r^2 = 0.99); intact assemblages yielded a V_{max} of 2.31 ± 4.70 µgP/µgChl/d and K_m of 23.44 ± 85.59 µgP/L (r^2 = 0.65). For long time periods the disturbed assemblages yielded a V_{max} of 8.42 ± 16.61 µgP/µgChl/d and K_m of 1236.55 ± 2831.18 µgP/L (r^2 = 0.99); intact assemblages yielded a V_{max} of 1.11 ± 1.25 µgP/µgChl/d and K_m of 653.45 ± 960.90 µgP/L (r^2 = 0.99). Additionally, we tested the soil nutrient uptake formulation of Barber (1995) for benthic biofilms by estimating C_{min}, the minimum [P] required for uptake to transpire. The Thellier plots used to estimate C_{min} yielded a [P] of 6.80 and 9.95 µg/L for the disturbed and intact biofilm treatments, respectively (Fig. 2).
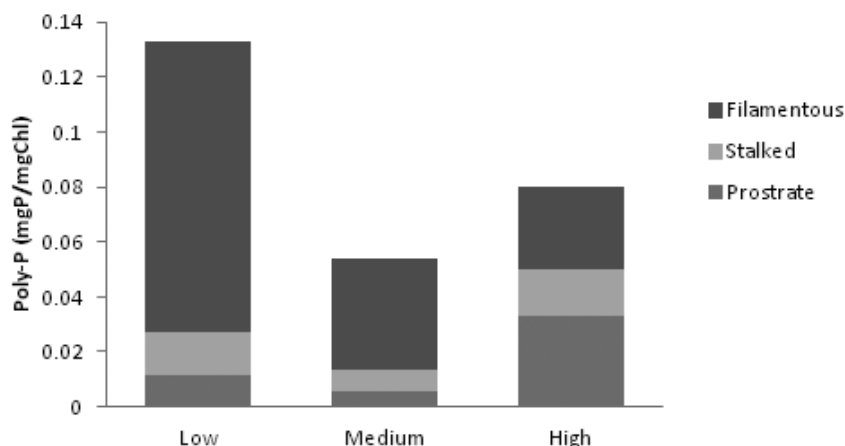
Fig. 1. Stacked column chart of benthic poly-P (mgP/mgChl) vs. disruption treatment according to growth form. Estimates were made by multiplying mean poly-P concentration for each disturbance treatment by the mean percent growth form from each physiognomic classification.
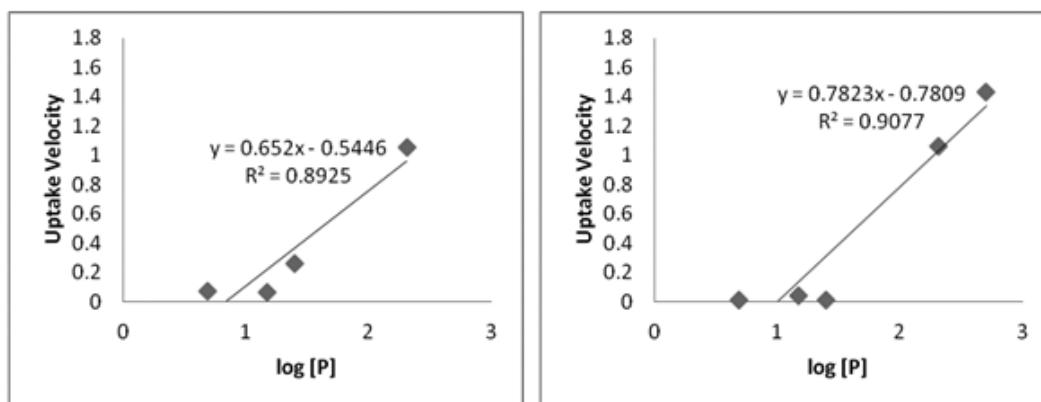

Fig. 2. Thellier plots showing uptake velocity (μgP/μgChl/d) versus log [P] (μgP/L) used to estimate C_{min} for disturbed (left) and intact (right) biofilm assemblages. The fitted linear regression intercepts the log [P] axis at the logarithmic threshold concentration (Aubriot et al. 2000).

Discussion

Measurements made between benthic biofilms subject to physical disturbance (abrasion) and undisturbed intact biofilms showed that single time point estimates of P uptake did not vary significantly between the treatments. These findings indicate that disturbance does not negatively affect the ability of biofilms to assimilate P during brief (≤30 min) single time-course experiments. While previous research has shown that metabolic responses differ depending on the units of normalization (e.g., Rosemond et al. 1993), we found here that both Chl and area-specific P-uptake rates were similar between the disturbance treatments, indicating robustness of our data. Moreover, our use of standardized substrates in an effort to remove variability in areal estimates and reduce sampling variability was likely effective (Lamberti and Resh 1985).

While uptake was estimated through direct incorporation of tracer into abraded biofilm microbes (on filter) in disturbed treatments and inferred through tracer loss from water overlying intact biofilms in undisturbed treatments, both methods seemed to be practical and comparable during this experimental period. A meta-analysis on P uptake in aquatic microbes supports this finding because experiments that derive uptake from water were comparable with those that derive uptake from direct analyses (Price and Carrick 2011). Further, our data here lend credence to the numerous experiments that investigate benthic microbial physiologic responses post-disturbance (e.g., Bothwell 1985, Reuter et al. 1986, Scinto and Reddy 2003).

The M-M parameter estimates did show relative differences when split by short and long time samplings, with the shorter yielding higher V_{max}; this finding corresponds to earlier research and likely indicates initial transport versus assimilation kinetics (Flynn 1998, Price and Carrick 2011). In fact, the rapid uptake revealed during our short time period (i.e., 5–12 min) is directly in

line with Taft et al. (1975), who found that initial rapid P uptake (attributed to membrane transport) declined after 15–60 min in phytoplankton in the Chesapeake Bay estuary. Our K_m estimates were similar to those reported for freshwater periphyton (508 µgP/L; Scinto and Reddy 2003). The rather large variations that we reported in abraded and intact assemblage P uptake was not surprising. Dodds (2003) compiled a range of P uptake rates for periphyton from natural and constructed systems and found variation more than 5 orders of magnitude. However, low replication of our individual experiments precluded more definitive statistical analysis.

While our disturbance design was more qualitative in nature, we did attempt to quantify relationships between our applied treatments and hydraulic disturbance. We estimated the percentage of biomass (as Chl) lost in each disturbance treatment by comparing biomass removed to average ambient biomass. These values were then compared to the percentage of biomass lost over various levels of shear stress following Biggs and Thomsen (1995) to approximate a relationship between our disturbance treatments and hydraulic shear magnitudes. Similar to Biggs and Thomsen (1995), *Melosira varians* was a dominant taxon in all of our applied disturbance treatments, and the estimated velocity at our site (0.43 m/s), approximated from water flow (0.60 m³/s) and stream area (1.39 m²) was within the ambient velocity range they cite.

Comparable inherent properties between the studies facilitate inferences. That said, our low, medium, and high disturbance treatments corresponded to mean shear stresses of 5.42 ± 1.90 (SD), 41.71 ± 24.82, and 67.36 ± 55.39 N/m², respectively. Despite this gradient of shear stress among the treatments, no level of imposed disturbance caused physiological changes in the microbes removed from the biofilms (as estimated by CFC). This indicates that, during brief incubation periods, there are no obvious deleterious effects arresting or impairing the rate of electron flow between photosystem II and photosystem I and, by extension, the ability of the disturbed biofilms to assimilate P (e.g., Rueter and Ades 1987). Viable cells sloughing from periphyton (benthic biofilms) have been observed before through microscopic examination (Naiman and Sibert 1978), and as stream benthic microbial biofilms are continually subject to the abrasive effects of suspended solids and bed sediments and consequently periodic sloughing losses (Biggs and Close 1989), it seems reasonable that they have adapted mechanisms to remain viable during such processes.

Our growth form recovery results from the increasing levels of disturbance study support those found elsewhere; specifically, nonfilamentous taxa in the biofilms showed the most resistance to the applied disturbance while filamentous taxa showed the least (Biggs and Thomsen 1995). For instance, organisms that grow prostrate to a substrate (e.g., *Achnanthes*) are naturally more resistant to dislodgment by shear and were, as expected, recovered only in high disturbance treatments. Such differential disturbance effects on microbial growth forms have implications for niche partitioning, and as a result, nutrient uptake and storage capacity (Cardinale 2011).

The quantity of biologically stored P (poly-P) varied among layers (growth form) within the biofilm with the highest concentration found in top (filamentous forms; 64%) and lower for both middle (stalked forms; 16%) and bottom (prostrate forms; 20%). Cells can store a large amount of P in poly-P granules (Jacobson and Halman 1982), and immediate accumulation in microbes has been observed during P surplus (Casadevall et al. 1985, Zeng and Wang 2009). Higher poly-P concentrations in the low disturbance treatment suggest that surplus P is available to filamentous forms in the biofilm. This was expected because filamentous microbes in a biofilm generally have greater biomass exposed to overlying waters where nutrients may be more bioavailable and further supports work showing that access to phosphate supplies from the water depends on the position of microbial cells in the biofilm (see Burkholder et al. 1990).

While our experiment somewhat suffered from imprecision in segregating the biofilm matrix (distinct growth forms intersected across disturbance treatments), our approach nonetheless allowed us to quantitatively estimate where P is stored. Because there is little direct data on nutritional content relative to spatial arrangement in a biofilm (Murdock et al. 2010), our research here thus forwards valuable, albeit coarse, data on poly-P according to successional growth forms in the complex matrix. Because poly-P can affect uptake and incorporation of P into aquatic microbes (Cotner and Wetzel 1992), understanding nutrient storage in varying strata of growing biofilms could help direct models predicting uptake capacity and downstream particulate P transports in sloughed material.

While we found that biofilms subject to both formaldehyde and glutaraldehyde treatments showed significantly reduced uptake rates compared to controls, the data suggest that abiotic uptake of P could be important in streams like the one studied here. These estimates for the abiotic fraction of uptake did show considerable P sorption (40% of total) and were comparable to previous literature. For example, Aldridge et al. (2010) found that abiotic interception accounted for more than 70% of the total P uptake by epilithic communities across a gradient of unmodified and modified streams. Our abiotic sorption estimate may be artificially elevated due to the absence of flow in our experiments. That is, a key mechanism of

abiotic P retention includes adsorption by sediments (Reddy et al. 1999), and therefore in a flowing system that specific mechanism would be diminished due to added transport and fewer opportunities for nutrient-sediment encounters (Triska et al. 1989).

Abiotic uptake is also dependent on biogeochemistry and sediment composition of the stream (Stone and English 1993). Here we measured a single system, but an analysis of abiotic uptake across spatial and temporal gradients should be further considered to refine models (Dodds et al. 2002). Despite this need, Price and Carrick (2011) found that only 32% of uptake experiments factor an abiotic fraction of uptake into analysis; we would therefore suggest this be performed on a more regular basis to avoid overestimation of biotic uptake rates. Further reason to address abiotic sorption is that some fraction of that sorbed P may ultimately desorb and become available for biotic uptake. Because P has multiple particulate phases, inclusive mathematical models should consider processes like adsorption and desorption to quantify the effects of nutrient inputs on system dynamics (Guillaud et al. 2000).

Concentrations of substrate below which cells cannot acquire nutrients reflect important and potentially ecologically meaningful threshold concentrations (Istvánovics and Herodek 1995). Due to energetic constraints, biotic uptake is then only possible when external [P] surpasses this threshold, or C_{min}, which in P-limited communities has been found to be in the nanomolar range (Falkner et al. 1989). Our values averaged 219 nM for the disturbed biofilm treatment and 321 nM for the intact treatment and thus seem to be within ranges previously reported. Previous work has suggested that the threshold concentration in P-deficient lakes is close to the ambient P concentration (lstvánovics and Herodek 1995).

Biofilms for this experiment were retrieved from an upstream site in Spring Creek, Pennsylvania, where orthophosphate concentrations are <10 µg/L and biofilm nutrient ratios indicate P limitation (Godwin and Carrick 2008); these conditions therefore provide further creditability to our results. Nevertheless, rapid nutrient cycling within biofilms (Riber and Wetzel 1987, Mulholland et al. 1994) may essentially act to insulate microbes and reduce dependency on bulk surface water nutrients; in such circumstances, C_{min} may be an extraneous parameter. Lastly, given that the magnitude of the C_{min} estimates are so low (nM), including it in the M-M model would not likely alter the shape of the simple rectangular hyperbolic (Istvánovics et al. 1993) in this set of experiments. Thus, our results seem to indicate that while C_{min} parameter estimates can be practical, their determination and usage in M-M models may not fundamentally alter benthic biofilm kinetic rate estimates.

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