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Seasonal shifts in the importance of bottom–up and top–down factors on stream periphyton community structure

Whitney S. Beck, David W. Markman, Isabella A. Oleksy, M. Holliday Lafferty and N. LeRoy Poff

W. S. Beck (http://orcid.org/0000-0002-0306-6086) (whitney.beck@colostate.edu), I. A. Oleksy, M. H. Lafferty and N. L. Poff, Graduate Degree Program in Ecology, 1878 Campus Delivery, Colorado State Univ., Fort Collins, CO 80526, USA. IAO also at: Dept of Ecosystem Science and Sustainability and Natural Resources Ecology Lab, Colorado State Univ., Fort Collins, CO, USA. – D. W. Markman, WSB, MHL and NLP also at: Dept of Biology, Colorado State Univ., Fort Collins, CO, USA. NLP also at: Inst. for Applied Ecology, Univ. of Canberra, ACT, Canberra, Australia.

We examined the importance of temporal variability in top–down and bottom–up effects on the accumulation of stream periphyton, which are complex associations of autotrophic and heterotrophic microorganisms. Periphyton contributes to primary production and nutrient cycling and serves as a food resource for herbivores (grazers). Periphyton growth is often limited by the availability of nitrogen and phosphorus, and biomass can be controlled by grazers. In this study we experimentally manipulated nutrients and grazers simultaneously to determine the relative contribution of bottom–up and top–down controls on periphyton over time. We used nutrient diffusing substrates to regulate nutrient concentrations and an underwater electric field to exclude grazing insects in three sequential 16–17 day experiments from August to October in montane Colorado, USA. We measured algal biomass, periphyton organic mass, and algal community composition in each experiment and determined densities of streambed insect species, including grazers. Phosphorus was the primary limiting nutrient for algal biomass, but it did not influence periphyton organic mass across all experiments. Effects of nutrient additions on algal biomass and community composition decreased between August and October. Grazed substrates supported reduced periphyton biomass only in the first experiment, corresponding to high benthic abundances of a dominant mayfly grazer (*Rhithrogena* spp.). Grazed substrates in the first experiment also showed altered algal community composition with reduced diatom relative abundances, presumably in response to selective grazing. We showed that top–down grazing effects were strongest in late summer when grazers were abundant. The effects of phosphorus additions on algal biomass likely decreased over time because temperature became more limiting to growth than nutrients, and because reduced current velocity decreased nutrient uptake rates. These results suggest that investigators should proceed with caution when extending findings based on short-term experiments. Furthermore, these results support the need for additional seasonal-scale field research in stream ecology.

Keywords: community ecology, herbivory, resources

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Introduction

Streambed periphyton is a complex association of autotrophic and heterotrophic microbes that can facilitate stream nutrient cycling (Battin et al. 2003) and provide a food source for primary consumers (Feminella and Hawkins 1995). It is critical to understand factors that regulate the biomass and composition of periphyton because of its important role in stream food webs and ecosystem functioning. Furthermore, stream periphyton serves as a water quality indicator because these species-rich communities respond rapidly to environmental changes (Stevenson 2014), and nuisance periphyton blooms may impose significant burdens on human health and ecosystems which requires management intervention to reduce biomass levels (Carpenter et al. 1998). A wealth of research has shown that periphyton can be regulated by both bottom–up and top–down factors (Francoeur 2001, Hillebrand 2002, 2009, Elser et al. 2007), although the strength of periphyton responses to resource additions and herbivory is influenced by stream context. Factors that change over time such as disturbance, nutrients, temperature, light and predator and prey densities may all influence the strength of bottom–up and top–down forces in natural ecosystems (Power 1992), thereby limiting the temporal scope of inference for many studies. Experiments and conceptual models from terrestrial (Hunter and Price 1992, Boyer et al. 2003, Gratton and Denno 2003) coastal marine (Thompson et al. 2008, Whalen 2013), and lentic systems (Weisse 1991) have incorporated temporal heterogeneity when determining the strength of bottom–up and top–down factors, but experiments from stream systems have rarely considered temporal variation in these factors.

Studies focusing on bottom–up effects on periphyton have added nutrients to streams, streamside channels, or mesocosms and measured responses of algal biomass (Fairchild et al. 1985), fungal biomass (Tank and Dodds 2003), bacterial abundance (Hoch 2008), and periphyton organic mass (Bechtold et al. 2012). Regardless of the microbial group being considered, periphyton growth is commonly limited by nitrogen (N) and phosphorus (P) availability in stream ecosystems (Francoeur 2001, Elser et al. 2007, Beck et al. 2017). Furthermore, nutrient availability alters periphyton community composition by mediating microbial competitive (Brown et al. 1981) and facilitative (Lang et al. 2012) interactions.

Periphyton responses to nutrients may also be highly dependent on variability in stream environmental conditions such as light, temperature and current velocity (Beck et al. 2017). Nutrient diffusing substrates (NDS) are often used to experimentally test algal responses to N and P additions by providing artificial colonization surfaces for periphyton (Fairchild et al. 1985, Francoeur 2001), and NDS have been used to demonstrate the importance of stream context. For instance, NDS experiments showed enhanced algal responses to a limiting nutrient (N) as stream light levels increased (Taulbee et al. 2005). Furthermore, seasonal manipulations of NDS have demonstrated higher levels of algal nutrient limitation in summer, most likely because of the warmer temperatures (Francoeur et al. 1999). These studies show that under some conditions, light or temperature can be more limiting to algal growth than nutrients. Finally, NDS have shown that stream current velocity can increase algal biomass responses to limiting nutrients (Hoch 2008) because of increased nutrient uptake with faster current (Borchardt et al. 1994).

Top–down control of periphyton by grazers such as aquatic insects and snails has also been demonstrated in many laboratory and field studies (Feminella and Hawkins 1995, Lamberti et al. 1995). Indeed, a meta-analysis found that grazer removal had a stronger positive effect on periphyton biomass than nutrient additions, although both effect sizes were significant (Hillebrand 2002). Grazers consume periphyton but may also cause non-consumptive biomass losses through physical disruption of periphyton communities (Eichenberger and Schlatter 1978, Lamberti et al. 1995). Furthermore, grazers have been shown to change algal community composition by selectively removing palatable diatoms (Rosemond et al. 1993) or overstory taxa (Feminella and Hawkins 1995), depending on the morphological traits of the grazers and growth forms of periphyton communities being studied (Steinman 1996).

Stream biological and environmental conditions such as grazer densities, predator densities, temperature and current velocity all vary over time and can substantially influence top–down control of periphyton by grazers. In field experiments, higher grazer abundances are linked to higher periphyton consumption rates (Hillebrand 2009), but grazer abundances change over time based on species' phenologies and grazing rates may decrease with predator abundances (Lourenço-Amorim et al. 2014). Temperature increases metabolic rates of grazers and may lead to higher periphyton consumption rates, as has been demonstrated across a wide range of laboratory and field experiments in lentic, lotic and marine systems (Hillebrand 2009). Finally, consumption rates may depend on how the grazer of interest responds to variation in stream current velocity (Poff et al. 2003).

A number of studies have also quantified periphyton responses to interactions between bottom–up and top–down factors (reviewed by Hillebrand 2002), but rarely have these interactions been investigated over time. Seasonal changes incorporate largely predictable shifts in environmental conditions that are likely to affect bottom–up and top–down influences on periphyton, but only one previous field study has examined the seasonal changes in resource and grazer regulation of periphyton. Rosemond et al. (2000) held grazing snail densities constant in experimental streamside channels and found that snails significantly reduced periphyton biomass and altered periphyton community composition across three seasons. Furthermore, resource additions generally only influenced periphyton structure when grazers were removed. The interaction between nutrients and

grazers in regulating periphyton biomass and community composition has not previously been examined with in-stream experiments that account for seasonal changes in grazer densities. Yet these seasonal changes are likely to be important, as studies from lake (Weisse 1991) and tidal systems (Thompson et al. 2008) have shown seasonally-variable top–down pressure on phytoplankton because of changes in grazer abundances. Additionally, research has shown that seasonal changes in resource quality and predator abundances influence the strength of bottom–up and top–down control on herbivores in grassland systems (Boyer et al. 2003, Gratton and Denno 2003).

In this study, we sought to understand how seasonal shifts in abiotic limiting factors and insect grazer abundance affect the relative importance of top–down and bottom–up factors on periphyton community structure in a temperate mountain stream. We completed a series of in-stream experiments from summer to early fall, using NDS to add nutrients (Fairchild et al. 1985, Tank et al. 2017) and underwater electric fields to exclude grazers (Pringle and Blake 1994, Opsahl et al. 2003, Moulton et al. 2004, Lourenço-Amorim et al. 2014). We measured multiple periphyton responses to these treatments including algal biomass, periphyton organic mass, an autotrophic index (AI), and algal community abundances of chlorophytes (green algae), bacillariophytes (diatoms), and cyanobacteria.

Methods

Study site and design

We completed experiments in the South Fork Poudre River at the Colorado State University Mountain Campus (40.57°N, −105.59°W), a low-order stream with an elevation of 2740 m. We selected an open-canopy study reach with sand, gravel and cobble substrate. Three experiments were deployed sequentially in the same reach: 12–28 August (Exp 1), 1–17 September (Exp 2), and 17 September–4 October (Exp 3) of 2017. During each experiment, we employed a split-plot design and designated two replicate grazer exclusion plots and two replicate control plots (the whole plot factor) with light, velocity, and depth conditions that were as homogeneous as possible. Each plot contained six replicate vials of four nutrient treatments (the sub-plot factor).

Nutrient addition treatments

We constructed NDS (Tank et al. 2017) by filling 30 ml plastic vials with either 2% agar (control treatment), agar + 0.5 M NaNO$_3$ (N treatment), agar + 0.05 M KH$_2$PO$_4$ + 0.05 M K$_2$HPO$_4$ (P treatment), or agar + all three nutrient chemicals (NP treatment). Two types of phosphate were used to create an agar pH that was close to neutral, to avoid any confounding influences of pH alterations on periphyton (Beck and Hall 2018). Individual vials were capped with fritted

glass discs (5.7 cm²) and randomly attached to three plastic L-bars so that each plot contained three parallel L-bars holding six replicates of each of the four nutrient treatment types (Fig. 1). The L-bars were submerged and anchored to paving stones (15 × 22 cm) using zip-ties.

Electrical exclusion of grazers

To measure the effect of aquatic insect grazers on periphyton communities, we constructed a solar-powered, battery-operated electrical exclusion system (Fig. 1) modified from Moulton et al. (2004) and Lourenço-Amorim et al. (2014). A 100-watt solar panel was wired in parallel to a 10 amp rated charge regulator using insulated cables. The charge regulator supplied and regulated the charge of two 12-volt, 35 amp-hour, deep-cycle sealed lead acid batteries which were wired in parallel using 8 gauge insulated wire repurposed from automotive jumper cables. Batteries supplied power to an electric fence energizer, which provided 6700 volts at 500 ohms with a maximum output of 6 Joules. In accordance with Ohm's Law, a high-voltage energizer was necessary to account for low stream water conductivity (Utz et al. 2017) and the small body size of the stream grazers to be excluded (Lourenço-Amorim et al. 2014). Two 12-m lengths of insulated 12.5-gauge steel wire were connected to the 'active' (positive) terminal of the fence energizer and two identical lengths of wire were connected to the 'ground' (negative/relative ground) terminal. The opposite end of each wire was spliced to 12-gauge uninsulated copper wire using specialized wirenuts to avoid galvanic corrosion between dissimilar metals. Splice connections were filled with waterproof dielectric grease prior to sealing with heat shrink tubing to ensure water resistance.

Rectangular exclosures were created on the streambed immediately surrounding the treatment vials using the uninsulated copper wires originating from the positive terminal of the fence energizer. Plastic tent stakes were used to maintain the rectangular configuration of the exclosure. The uninsulated copper wires originating from the ground terminal of the fence energizer were anchored ~1 cm above the center L-bar in each electrified plot to generate an electrical gradient covering the entire treatment plot (Fig. 1). The electrical exclosures measured 45 × 30 cm and electrified and control plots were 1–2 m apart to prevent interaction. Previous research has shown that electrical exclosures do not significantly influence periphyton growth rates (Brown et al. 2000). To ensure the fence was excluding a broad range of insect size classes, we observed the behavior of Ephemeroptera, Plecoptera and Coleoptera individuals when exposed to the electrified exclosures. Twitching and contractions were observed across all tested insect orders during electric pulses. Insects were unaffected if they were greater than ~5 cm outside the exclosure. The fence energizer delivered an electric pulse every 2.5 s during the day and every 1.5 s at night, with more frequent pulses at night to account for increased insect drifting (Waters 1972).

Terrestrial | Aquatic

Water flow

Solar Panel
− +

Charge Regulator
− +

12V Battery
− +

12V Battery
− +

Fence Energizer
− +

Electrified

Electrified

- ─·─ Positive insulated
- ········· Positive bare
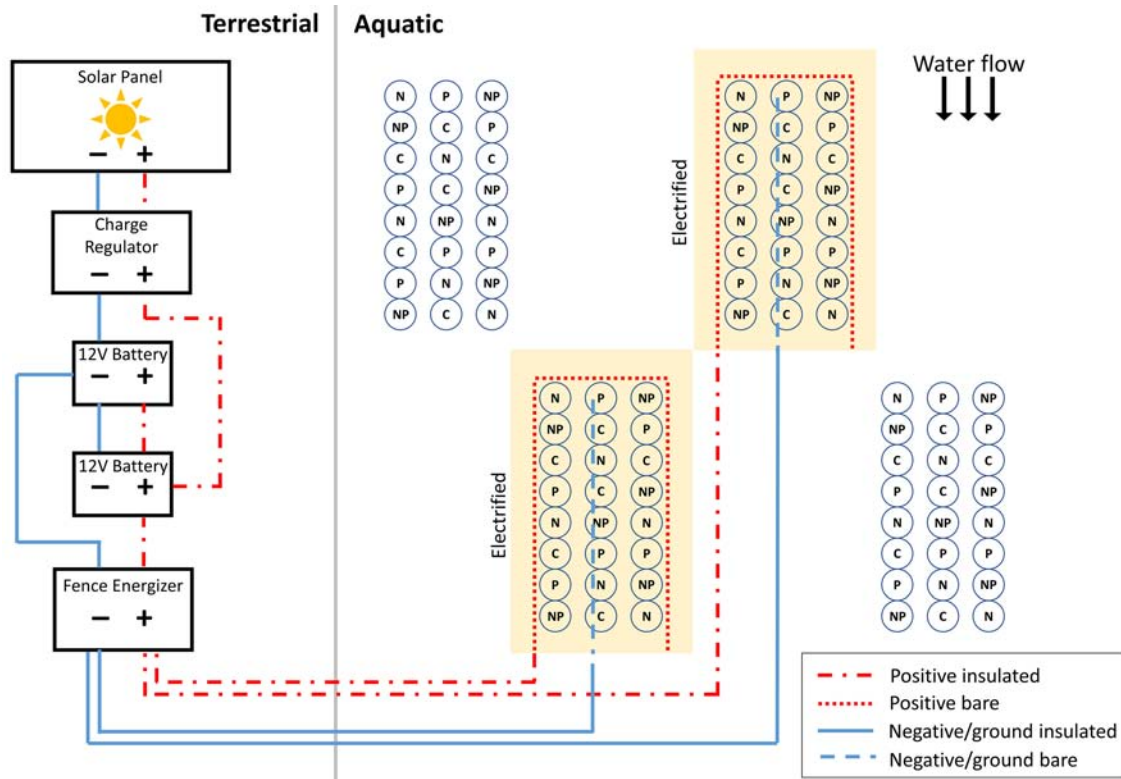- ──── Negative/ground insulated
- ─ ─ ─ Negative/ground bare

Figure 1. Conceptual schematic for electrical exclusion of stream grazers. An exclusionary zone (box surrounding two center experimental plots) was created by electrical current moving from bare wires carrying positive charge to bare wires designated as negative/ground. Insulated wires were connected to the positive or negative/ground terminals of each device as indicated in the figure key. Plots were staggered to avoid electrical or nutrient interference between plots. Diagram not shown to scale.

Environmental variables

At the beginning and end of each experiment, we measured fine-scale flow velocity immediately above NDS discs at three points on each L-bar using a 2.5 cm-scale MiniWater 20 flow meter. At the beginning of each experiment, we also measured canopy cover at each plot using a densiometer and collected duplicate filtered and unfiltered water samples from one point upstream. We filtered stream water through type A/E glass fiber filters (0.45 µm retention) into 60 ml bottles and analyzed samples for NO_3^- using the Cd reduction method (U.S. EPA Method 353.2 1993) and orthophosphate using the ascorbic acid method (Murphy and Riley 1962) on an autoanalyzer. We analyzed unfiltered stream water for total N using a TOC/TN analyzer. During each experiment, we took at least six underwater photographs per plot on three different dates to investigate algal disc colonization by invertebrates. At the end of each experiment, we measured streamflow using a Marsh McBirney meter and measured pH, conductivity and temperature using a multimeter and probe. Finally, at the end of each experiment we collected two Hess samples of aquatic invertebrates from the streambed surrounding the experimental plots to characterize community composition. In the laboratory, we used a dissecting microscope to separate macroinvertebrates from the substrate. The invertebrates were then identified to the lowest taxonomic unit needed to

assign functional traits (Poff et al. 2006), which was genus for most individuals. Over 1000 individuals were identified from 31 taxa groups.

Response variables and analysis

At the end of each experiment, we collected NDS discs and stored them at −20°C until chlorophyll a analysis within 30 days. We extracted chlorophyll a (a measure of algal biomass) directly from four replicate discs using 90% buffered ethanol, and we quantified the pigment mass using a hand-held fluorometer with an acidification correction (U.S. EPA 1997).

After chlorophyll a analysis, we allowed the liquid from the extraction slurry and NDS discs to evaporate in weigh boats under a fume hood then measured ash-free dry mass (AFDM, APHA 2005) using all remaining material (Bechtold et al. 2012). Ash-free dry mass incorporates not just algal biomass, but also the biomass of heterotrophic microbes and detritus from periphyton. We dried the weigh boat contents (including the NDS discs and particulates from the slurry) for 48 h at 50°C, measured their initial masses, and combusted them at 500°C for one hour. We then rehydrated the weigh boat contents with deionized water, dried them for another 48 h at 50°C, and measured their final masses. The rehydration step allowed us to account for water that was lost from clay

particles during combustion. The difference in initial and final masses was calculated as AFDM, and we calculated an autotrophic index (AI) as the ratio of chlorophyll a to AFDM (APHA 2005). Lower values of the index indicated a higher proportion of autotrophy in the microbial community (Bechtold et al. 2012). We also used chlorophyll a values and periphyton AFDM values to calculate separate log response ratios (LRR, a measure of effect size) for electrical exclusions and nutrient additions:

$$LRR_E = \ln \frac{\text{electric mean}}{\text{control mean}} \qquad (1)$$

$$LRR_N = \ln \frac{\text{nutrient mean}}{\text{control mean}} \qquad (2)$$

Treatments had a positive effect on the response variable of interest when the LRR was greater than zero and a negative effect when the LRR was less than zero (Tank and Dodds 2003).

We used the remaining two replicate discs to determine algal community composition using a UPLC-UV-MS system, modifying the procedure of Fu et al. (2012). Previous studies have shown strong agreement between LC-based measurements and microscopic determinations of algal community composition (Wright et al. 1996, Schlüter et al. 2006). Variation in environmental factors like light levels can obscure relationships between the two measurement methods (Havens et al. 1999), but environmental conditions among our plots were highly standardized compared to studies of lake and ocean phytoplankton communities. We measured three target pigments to capture variability in algal community composition: chlorophyll b for chlorophytes (green algae), fucoxanthin for bacillariophytes (diatoms), and myxoxanthophyll for cyanobacteria (Leavitt and Hodgson 2002). Hereafter, we use the algal group name rather than the pigment name. We also measured total chlorophyll a as the sum of chlorophyll a and three primary breakdown products in our samples, including chlorophyll a′, pheophytin a, and pheophytin a′ (Sartory 1985). We extracted algal pigments directly from discs using an 85:10:5 by volume acetone:methanol:water solution (Steinman et al. 2017). We filtered the extractant through 0.22 μm nylon syringe filters and dried the solution under N_2 gas until no liquid remained (Steinman et al. 2017). We then resuspended the pigments with a 1:1 by volume acetonitrile:MTBE solution (Fu et al. 2012). We used a mass spectrometer to identify pigments based on their known masses, which were confirmed with pigment standards. During 6-min runs for each sample, we used an Acquity ultra performance liquid chromatography (UPLC®) system with a tunable UV detector to separate pigment compounds from sample mixtures and measure their intensities (Fu et al. 2012). We integrated UV curve areas using Compass Hystar data software. We used calibration curves from pigment standards to convert UV areas to masses

(Leavitt and Hodgson 2002), then standardized community composition masses by total biomass as follows:

$$\text{Chlorophytes} = \frac{\text{chlorophyte mass}}{\text{total chlorophyll } a \text{ mass}} \qquad (3)$$

$$\text{Bacillariophytes} = \frac{\text{bacillariophyte mass}}{\text{total chlorophyll } a \text{ mass}} \qquad (4)$$

$$\text{Cyanobacteria} = \frac{\text{cyanobacteria mass}}{\text{total chlorophyll } a \text{ mass}} \qquad (5)$$

Statistical analyses

We performed statistical analyses in R ver. 3.5.0 (<www.r-project.org>). To analyze data from the split plot design, we separated the analyses by experiment and used ANOVAs with electricity and nutrients as factors along with plot as a random block to quantify treatment effects on algal biomass (chlorophyll a), periphyton AFDM and AI. We used ANOVAs with electricity and nutrients as factors but no random block for the algal community response metrics, because we sampled fewer replicates and therefore had lower statistical power in those models. Finally, we used one-way ANOVAs with experiment as a predictor and aquatic insect order abundances as response variables to test for changes in streambed insects. When there was a particularly abundant family within an order, we used that family as a separate response variable. For all models, we used contrasts of least-squared means with Tukey-adjusted p-values to determine significant differences among factors ($\alpha = 0.05$).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.q436v45> (Beck et al. 2018).

Results

Both nutrients and insect grazing regulated periphyton biomass metrics and community composition, but the independent and interactive effects depended on the response variable and time period being considered. Figure 2 shows that nutrients had a significant effect on algal biomass in experiment 1 ($F_{3,55} = 127.503$, $p < 0.001$) and experiment 2 ($F_{3,56} = 53.534$, $p < 0.001$), with higher values on P and NP treatments than no-nutrient controls ($p < 0.001$). Nutrients also had a significant effect on algal biomass in experiment 3 ($F_{3,53} = 5.877$, $p = 0.002$), with NP treatments being marginally significantly higher than controls ($p = 0.068$). Nutrients influenced AI in experiment 2 ($F_{3,53} = 5.068$, $p = 0.003$), with reduced values and therefore higher autotrophy on P and NP treatments ($p = 0.011$ and $p = 0.018$). Periphyton AFDM did not respond to nutrients in any of the experiments ($p > 0.05$).
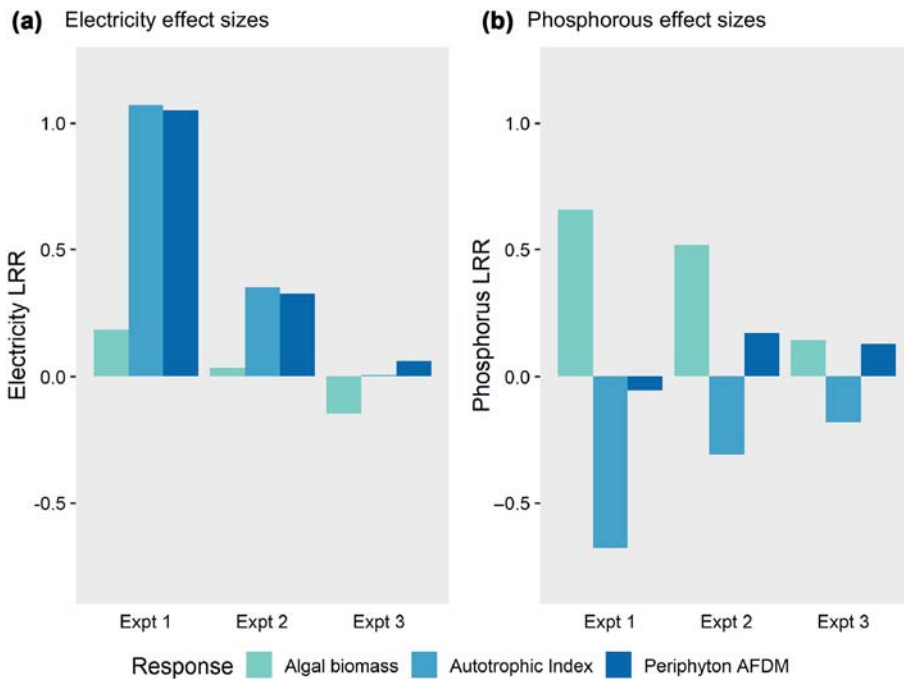
Figure 2. Experimental effect sizes (measured as log response ratios, LRRs, Eq. 1, 2) of electric and primary limiting nutrient (phosphorus) treatments calculated separately for algal biomass, periphyton ash-free dry mass (AFDM), and an autotrophic index (AI). Supplementary material Appendix 1 Fig. A1, A3 for statistical test results from untransformed values, showing a significant effect of electricity on AFDM and AI in the first experiment and a significant effect of P treatments on algal biomass in the first and second experiments. Table 1 for experimental time periods.

Algal community composition was also modified by nutrients, with strongest effects occurring earlier in the study (Fig. 3). In the first experiment, bacillariophytes ($F_{3,23} = 8.915$, $p < 0.001$) and cyanobacteria ($F_{3,21} = 8.389$, $p < 0.001$) were higher on the NP treatments ($p < 0.001$ for both). In the second experiment, nutrient treatment only influenced cyanobacteria ($F_{3,21} = 14.260$, $p < 0.001$), which was higher on NP treatments compared to no-nutrient controls ($p < 0.001$).
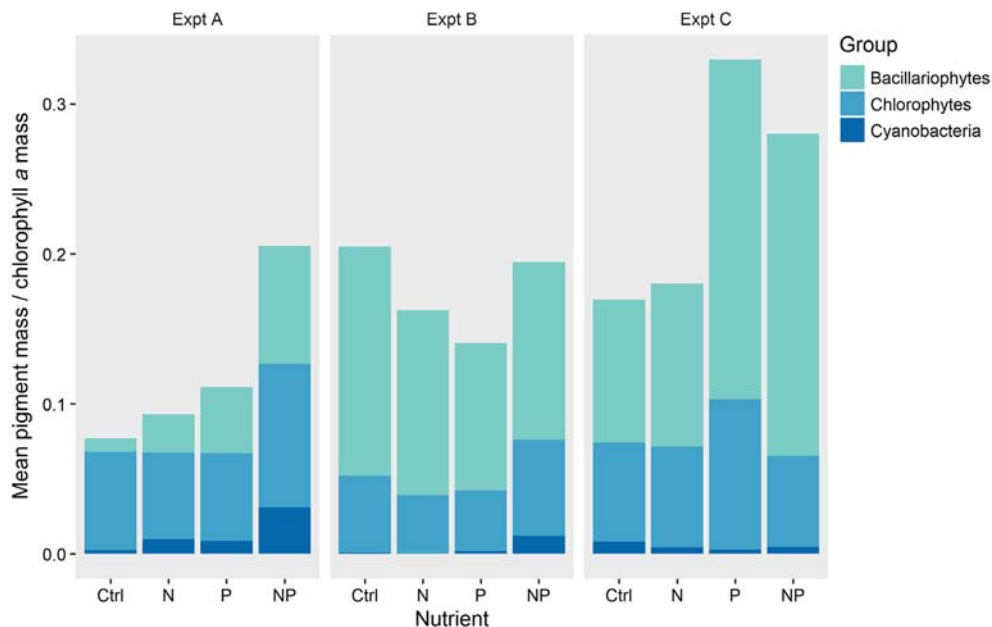

Figure 3. Mean bacillariophyte, chlorophyte, and cyanobacteria responses to nutrient treatments (Ctrl = control, N = nitrogen, P = phosphorus, NP = nitrogen and phosphorus) during three sequential experiments at the South Fork Poudre River. Supplementary material Appendix 1 Fig. A2 for variances and a summary of statistical tests.

No algal groups responded to nutrient treatments in the third experiment ($p > 0.05$).

The effects of grazing on periphyton biomass metrics and algal community composition were determined using electrical exclusion and were strongest in the first experiment (Fig. 2, 4). Periphyton AFDM and AI were significantly higher in electrical exclusion plots (i.e. reduced grazers) as compared to grazed controls ($F_{1,51} = 22.325$, $p = 0.0470$ and $F_{1,51} = 43.302$, $p < 0.001$), but algal biomass was not influenced by electricity ($p > 0.05$). Bacillariophytes were more abundant ($F_{1,23} = 6.396$, $p = 0.019$) on electricity treatments as compared to grazed controls. However, this pattern was not observed on N treatments, as we found a significant electricity × nutrient interaction for bacillariophytes ($F_{3,23} = 4.589$, $p = 0.012$). We found no main effect of electricity on periphyton biomass metrics or algal community composition in the second or third experiments ($p > 0.05$). However, we did find an interactive effect of electricity and nutrients on bacillariophytes in the third experiment ($F_{3,20} = 3.720$, $p = 0.028$), with higher relative abundances on grazed controls for all nutrient treatments except P.

We observed seasonal trends in the electricity and nutrient addition effect sizes as well as streambed grazer abundances. Algal biomass LRRs for both electricity and limiting nutrient (P) additions tended to decrease over time (Fig. 2), and periphyton AFDM LRRs for electricity also decreased over time. We found a marginally significant effect of experiment on heptageniid mayflies ($F_{2,3} = 7.775$, $p = 0.065$), with the first experiment having higher abundances compared to the second ($p = 0.074$) and third ($p = 0.095$) experiments. We found no effect of experiment on other aquatic insect groups.

Discussion

In this study, the strength of bottom–up and top–down factors on periphyton structure changed over time in a temperate mountain stream. This is the first demonstration of seasonal changes in these factors by an in-stream study (but see Rosemond et al. 2000 for a streamside mesocosm experiment), but it is likely a common phenomenon in seasonally-varying streams with substantial implications for generalizations about top–down and bottom–up drivers of periphyton dynamics. Our results add to a growing body of literature across a wide variety of ecosystems demonstrating temporal heterogeneity in the strength of bottom–up and top–down drivers (Weisse 1991, Boyer et al. 2003, Whalen et al. 2013). We also found that periphyton responded differently to electricity and nutrient treatments depending on the response variable being considered, which highlights the importance of treating periphyton as a heterogeneous microbial community in experimental studies. We outline the likely drivers of periphyton changes in our study, but also discuss how experimental design can influence both outcomes and appropriate scales of inference for stream field studies.

In the South Fork Poudre River, P was a primary driver of algal biomass accrual, and the effect of P additions changed over time. Pigments indicative of algal biomass
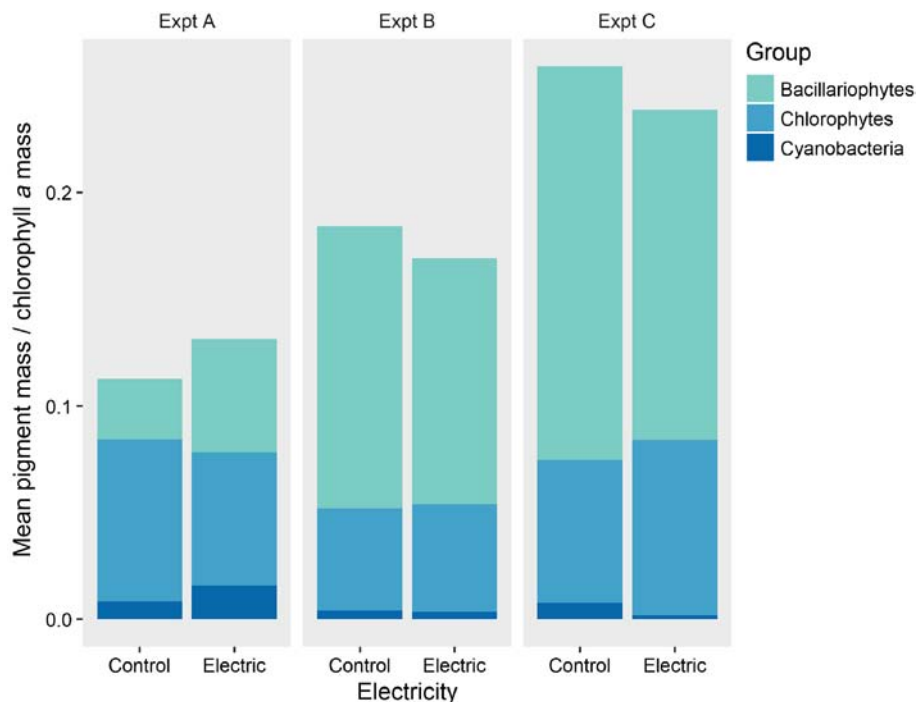

Figure 4. Mean bacillariophyte, chlorophyte, and cyanobacteria responses to electrical grazer exclusion treatments during three sequential experiments at the South Fork Poudre River. Supplementary material Appendix 1 Fig. A4 for variances and a summary of statistical tests.

and community composition responded to P and NP additions, but periphyton AFDM did not. It is likely that the heterotrophic component of the community was limited by another factor such as carbon (Bechtold et al. 2012), which we did not measure during this study. As a result, autotrophs generally comprised a higher proportion of the periphyton community as compared to heterotrophs on NDS P treatments. This pattern has been demonstrated in other studies as well, whereby limiting nutrient additions lead to a decoupling of heterotrophic and autotrophic components of periphyton (Scott et al. 2008). Specifically, it has commonly been hypothesized that heterotrophs are better competitors for P under limiting conditions (Brown et al. 1981), but autotrophs are expected to increase with P additions as we found in this experiment. In terms of algal community composition, we found that bacillariophytes and cyanobacteria increased on NP nutrient treatments during the first experiment, and cyanobacteria also increased on NP treatments in the second experiment. Bacillariophytes and cyanobacteria likely depended more on NP substrate additions as compared to chlorophytes. The chlorophytes in the South Fork Poudre River were dominated by filamentous taxa such as *Chaeotophera* spp., *Cladophora* spp., and *Spirogyra* spp. (Beck unpubl.), and these taxa had more access to water column nutrients as compared to the adnate (low stature) bacillariophytes and cyanobacteria (largely *Oscillatoria* spp.). Bacillariophytes in particular tend to be early successional colonizers (Peterson and Stevenson 1990) and may have thrived on the limiting nutrient substrates.

We observed temporal changes in both periphyton nutrient limitation and algal community composition which were likely related to changing environmental conditions. Particularly in high elevation and high latitude streams, algal growth may be limited by factors like temperature (Cross et al. 2015) and light levels (Gustina and Hoffmann 2000) in addition to N and P availability (Toetz and Payton 1999, Cardinale et al. 2009, Bowman et al. 2005). Temperatures cooled throughout the summer, as point temperature measurements decreased from 11.5°C in August to 6–7°C in September and October (Table 1). A study in the same watershed used water temperature logger data to show substantial cooling from August to October (Shah et al. 2017), which can decrease algal responses to nutrient additions (Francoeur et al. 1999). Decreased insolation has also been shown to reduce nutrient limitation in past experiments (Rosemond et al. 2000, Taulbee et al. 2005), but in our study seasonal decreases in insolation may have been balanced by decreases in water depth over time (Table 1) that likely increased light availability in the water column. Current velocity also decreased over time, which may have decreased nutrient uptake rates (Horner and Welch 1981, Borchardt et al. 1994) and led to weaker nutrient limitation. Additionally, the algal communities changed in response to these environmental conditions, specifically with chlorophytes decreasing and bacillariophytes increasing throughout the season, probably reflecting the higher tolerance of bacillariophytes to cooler temperatures (DeNicola 1996). Cyanobacteria also decreased

Table 1. Environmental conditions at the South Fork Poudre River, Colorado during three experiments from August 2017 to October 2017. Canopy cover and nutrient measurements were made at the beginning of each experiment, velocity and depth measurements were made at the beginning and end of each experiment, and all other measurements were made at the end of each experiment. Data with multiple measurements are reported as mean ± SE.

Expt	Dates	Temp (°C)	Conductivity (µS cm⁻¹)	Discharge (m³ s⁻¹)	Canopy (%)	Velocity (cm s⁻¹)	Depth (cm)	Nitrate-N (µg l⁻¹)	Phosphate-P (µg l⁻¹)	TN (µg l⁻¹)
1	8/12/17–8/28/17	11.5	20.92	2.32	7.28	20.42 ± 1.56	21.13 ± 1.87	85.9 ± 2.10	<detection	117.9 ± 1.9
2	9/1/17–9/17/17	5.8	27.3	0.42	4.75	16.25 ± 0.68	13.54 ± 1.29	58.10 ± 0.80	0.79 ± 0.29	103.71 ± 7.09
3	9/17/17–10/04/17	6.7	27.85	0.26	1.13	11.81 ± 0.97	17.08 ± 0.54	61.05 ± 0.65	<detection	107.15 ± 5.85

over time and always comprised a low proportion of the community because these taxa are intolerant of cool temperatures (DeNicola 1996). These results highlight how factors change seasonally and indicate that investigators should proceed with caution when extending nutrient limitation findings based on short-term experiments.

We also found that grazing treatments influenced most periphyton response metrics, and as the streambed grazer densities declined over time, so did the inferred strength of top–down control. In the first experiment, periphyton AFDM and the proportion of bacillariophytes were significantly reduced (Fig. 2, 4), and this corresponded to the highest abundances of mayfly grazers (Heptageniidae, primarily *Rhithrogena* spp.) on the streambed (Fig. 5). Although we did not observe aquatic insects on NDS surfaces during the day, many aquatic insects in our system (including heptageniid mayflies) commonly drift through the water column at night (Poff et al. 2006), and they could easily access the artificial substrates used to grow algae (Opsahl et al. 2003). Grazers can selectively feed on bacillariophytes (Rosemond et al. 1993, 2000), and grazers as well as non-grazers can cause non-consumptive losses of periphyton through physical disruption as they move over the substrate (Eichenberger and Schlatter 1978, Lamberti et al. 1995).

None of our response metrics were significantly influenced by insect grazers in the second and third experiments. Positive or neutral effects of grazers on periphyton biomass have been reported in other studies and likely occur because of indirect effects related to insect competition, nutrient cycling, or sediment and detritus removal (Hillebrand 2009). However, periphyton removal likely decreased over time because we found a marginal decrease in *Rhithrogena* spp. after the first experiment (despite low statistical power from n = 2 samples), which almost certainly emerged from the stream as adults (B. C. Kondratieff, Colorado State Univ. pers. comm.). Indeed, in an energetics study in a neighboring watershed, Carlisle (2002) found *Rhithrogena* spp. streambed abundances decreased from summer to fall, leading to an increased standing stock of algal biomass and decreased consumption of bacillariophytes. Studies from lentic and tidal systems have also demonstrated seasonal changes in grazer abundances that significantly decreased top–down pressure on phytoplankton (Weisse 1991, Thompson et al. 2008). For those insects remaining in the stream during our study, overall aquatic insect activity and metabolic rates may have decreased with cooling temperatures over time, contributing to reduced periphyton removal as has been shown across a wide variety of stream, lake and marine experiments (Hillebrand 2009). These results indicate that top–down control may be variable and is dependent on grazer community composition and environmental conditions that shift over time. However, it is important to consider that grazers may have influenced algal
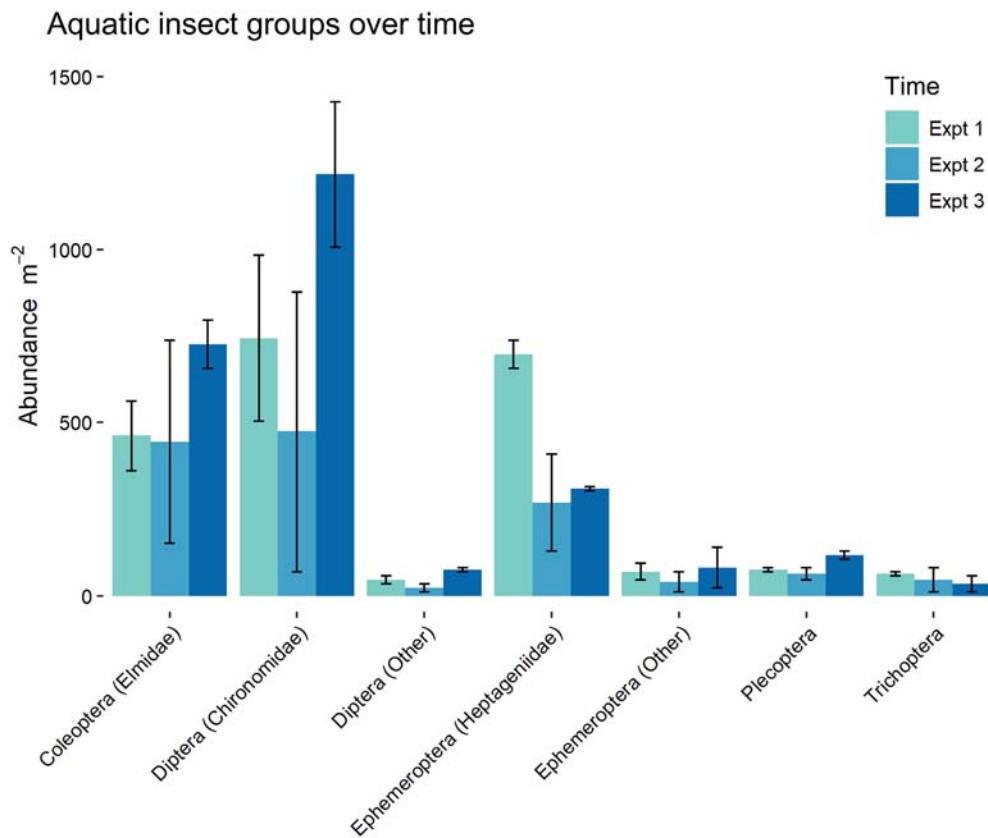

Figure 5. Streambed aquatic insect groups (mean ± SE) measured with two replicate Hess samples at the end of each of three sequential experiments in the South Fork Poudre River.

community composition at a finer scale than what was measured during this study, as grazers have been shown to select for palatable and accessible taxa even within bacillariophyte, chlorophyte or cyanobacteria communities (Steinman et al. 1992).

Past meta-analyses have found that experimental duration was an important factor influencing the relative strength of bottom–up and top–down effects on periphyton (Feminella and Hawkins 1995, Hillebrand 2002, 2009). The positive effect of nutrients on periphyton decreases with experimental duration, while the negative effect of grazing on periphyton increases with experimental duration (Hillebrand 2002, 2009). This is likely because more developed periphyton communities can internally recycle nutrients and rely less on external sources, but grazing rates increase with available periphyton biomass (Hillebrand 2002, 2009). In our study, nutrient additions had a stronger influence on periphyton biomass metrics than did grazer exclusions, and we saw no interaction between grazer removal and nutrient additions on periphyton biomass metrics. We completed relatively short periphyton colonization experiments because nutrient diffusion rates from NDS decline logarithmically over time (Rugenski et al. 2008). Nutrients would be expected to have strong control on periphyton biomass in the early successional communities that developed. Only one other experiment has used NDS in conjunction with electrical exclusions, finding that nutrients had a much larger effect than grazer exclusions on algal biomass in a similarly short, 15-day experiment (Lourenço-Amorim et al. 2014). However, it is important to consider that a longer experiment would require replenishing nutrients in the NDS agar. In addition, periphyton may surpass peak biomass in a longer experiment and begin to slough from growth surfaces (Biggs 1996), making it difficult to distinguish autogenic sloughing from treatment effects.

While any experimental design includes compromises, field experiments employing underwater electric fences allowed for the observation of complex dynamics between periphyton communities and temporally variable drivers like shifts in insect communities and background environmental conditions. Laboratory and streamside enclosure experiments are often not representative of in-stream conditions and tend to estimate higher grazing rates than field experiments (Feminella and Hawkins 1995, Hillebrand 2009). In contrast, in-stream experiments maintain natural insect densities and processes like drifting, emergence, predation, and abiotic variability which significantly influence grazing. While field experiments on herbivory may be challenging, electric fences have the advantage of excluding aquatic insects with small body sizes (Moulton et al. 2004) while also avoiding the effects of sedimentation or altered current velocity that may accompany other in-stream exclosure devices.

Conclusion

We found that the strength of bottom–up and top–down controls on periphyton decreased over time from summer to early fall in the South Fork Poudre River, which adds to the growing number of studies demonstrating temporal heterogeneity of top–down and bottom–up importance across terrestrial (Hunter and Price 1992, Boyer 2003, Gratton and Denno 2003), marine (Thompson et al. 2008, Whalen et al. 2013), and lentic ecosystems (Weisse 1991). Nutrient additions had a substantial effect on algal biomass and community composition, while grazer exclusions affected periphyton AFDM and algal community composition early in the season because of consumptive and non-consumptive biomass losses. We recommend that future experiments consider temporal variability when investigating bottom–up and top–down regulation of stream periphyton in natural stream ecosystems to better account for physical and biological controls on nutrient limitation and herbivory. In addition, management decisions based on stream periphyton experiments should carefully consider the limited temporal scope provided by individual experiments.

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