



Spatial Variability in Light Yields Colimitation of Primary Production by Both Light and Nutrients in a Forested Stream Ecosystem

Dana R. Warren,^{1,2*} Sarah M. Collins,³ Emily M. Purvis,⁴
Matthew J. Kaylor,² and Heather A. Bechtold⁵

¹Department of Forest Ecosystems and Society, Oregon State University, Richardson Hall, Corvallis, Oregon 97331, USA; ²Department of Fisheries and Wildlife, Oregon State University, Nash Hall, Corvallis, Oregon 97331, USA; ³Department of Fisheries and Wildlife, Michigan State University, 165 Natural Resources Building, East Lansing, Michigan 48824, USA; ⁴H.J. Andrews Long-term Ecological Research Program, Oregon State University, Blue River, Oregon 97413, USA; ⁵Department of Biological Sciences, East Campus Science Center, Lock Haven University of Pennsylvania, Lock Haven, Pennsylvania 17745, USA

ABSTRACT

Colimitation of primary production is increasingly recognized as a dominant process across aquatic and terrestrial ecosystems. In streams, both nutrient availability and light availability have been shown to independently limit primary production, but colimitation by both light and nutrients is rarely considered. We used a series of nutrient-diffusing substrates (NDS) bioassays deployed across a range of light availability conditions in a single-study stream over two summers to determine the light level at which the limiting factor for benthic periphyton accrual transitioned from light to nutrients. Stream periphyton accrual was nutrient-limited in high-light patches, and light-

limited in low-light patches, with the transition from being predominantly light-limited to being predominantly nutrient-limited occurring when daily light fluxes exceeded $3.5 \text{ mol m}^{-2} \text{ day}^{-1}$. We quantified light at each NDS bioassay location and at 5 m intervals throughout our two adjacent 160 m study reaches—one in structurally complex old-growth riparian forest and one bordered by more uniform second-growth forest. Although both reaches were colimited overall, the resource (light or nutrients) dominating limitation differed between the two riparian forest age/structure conditions. In the old-growth section, about three quarters of the reach was predominantly nutrient-limited, whereas in the second-growth reach only about a quarter of the streambed was nutrient-limited. In this stream, colimitation of benthic periphyton accrual by light and nutrients at the reach scale was an emergent property of the ecosystem that manifested as a result of high heterogeneity in riparian forest structure.

Key words: nutrient-diffusing substrate; nitrogen limitation; light limitation; colimitation; benthic primary production; HJ Andrews; riparian forest; stream light; habitat heterogeneity.

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*Corresponding author; e-mail: dana.warren@oregonstate.edu

INTRODUCTION

Primary production influences the processing, retention, and export of carbon and nutrients in an ecosystem; therefore, understanding the factors that limit primary production will enhance our understanding of how multiple components of an ecosystem are likely to respond to changing environmental conditions (Grimm and others 2013; Rosemond and others 2015; Vitousek and others 1997). The potential for a single-limiting factor to control gross primary production (GPP) and a wide range of associated ecosystem processes is a fundamental concept in ecology; however, recent syntheses have highlighted the importance and prevalence of colimitation (limitation of GPP by two or more factors together) rather than single-factor limitation at the community and ecosystem scale (Danger and others 2008; Elser and others 2007; Harpole and others 2011; Sperfeld and others 2012; Fay and others 2015). Colimitation at these larger community or ecosystem scales may occur through multiple pathways: (1) biological demand for both resources together by a given organism ("*simultaneous colimitation*"), (2) aggregate small-scale single-factor limitation within an ecosystem or community which manifests as colimitation at a larger scale ("*independent colimitation*"), or (3) alternating transitions in demand over short time periods between multiple resources ("*serial colimitation*") (per Harpole and others 2011). In aquatic ecosystems, much of the work on colimitation has focused on simultaneous colimitation at the patch scale (Marcarelli and Wurtsbaugh 2007; Johnson and others 2009; Tank and Dodds 2003), but at the ecosystem scale other mechanisms or types of colimitation may occur. Similarly most colimitation studies in freshwater aquatic ecology have focused on multiple nutrients, yet other resources can also limit primary production and recent studies increasingly suggest that these other resources (particularly light) warrant consideration as colimiting drivers of primary production (Hill and Fanta 2008; Kiffney 2008; Von Schiller and others 2007). In this study, we conducted an empirical assessment of nutrient limitation and light limitation in a headwater stream to explore how spatial heterogeneity in local light availability can create independent colimitation at a larger ecosystem scale.

In forested headwater streams, the two dominant abiotic resources that limit primary production are nutrients and light (Hill and others 1995; Larned 2010; Tank and Dodds 2003). Many studies have focused on nutrient limitation in streams, consid-

ering both single nutrient limitation and colimitation by multiple nutrients, primarily nitrogen and phosphorus (Bechtold and others 2012; Harpole and others 2011; Larned 2010; Johnson and others 2009; Tank and Dodds 2003). Light limitation has also been well-explored in forested headwater streams, and a number of studies have suggested that the importance of nutrient availability arises only after light limitation has been alleviated—a form of serial colimitation (Ambrose and others 2004; Bernhardt and Likens 2004; Carey and others 2007; Elsaholi and others 2011; Julian and others 2011; Rier and others 2014) but see (Mosisch and others 1999). In most cases, however, when light and nutrients are evaluated as drivers of stream primary production, discussions focus on identifying which individual factor is the dominant influence on stream GPP through a reach at a given time (Greenwood and Rosemond 2005; Rosemond and others 2000). Few studies have explicitly addressed colimitation by light and nutrients together (but see Carey and others 2007; Hill and Fanta 2008; Kiffney 2008). This is perhaps due to the common assumption in field studies that light is consistent at the stream reach scale. However, work exploring spatial dynamics of light in streams has demonstrated that light can be highly variable at small spatial scales and both the amount and spatial variability of stream light will change over time as riparian forests develop (Keeton and others 2007; Warren and others 2013), which could result in shifts in the amount of light versus nutrient limitation.

Research on stream nutrients has explicitly noted the potential importance of local variability in nutrient availability in creating areas of elevated processing—that is, biogeochemical hotspots (McClain and others 2003)—however, research on stream light has generally focused on broad reach-scale trends in solar flux with relatively few field studies considering local variability in light (Denicola and others 1992). Much of the *in situ* experimental research exploring the influence of light on stream primary production with associated food web and nutrient cycling dynamics in streams has focused on large differences or changes in light associated with the presence or absence of forests within the riparian area (Bilby and Bisson 1992; Hill and others 1995; Moslemi and others 2012; Noel and others 1986; Sabater and others 2000; Stone and Wallace 1998). These studies clearly demonstrate that dramatically increasing light in forested headwaters can increase primary production and nutrient uptake. Fewer studies have evaluated

primary production and nutrient dynamics across a gradient of light in streams but work to date suggests that even small changes in the amount of riparian shading within a stream reach can influence primary production and the cycling of nutrients in streams (Carey and others 2007; Finlay and others 2011; Hill and others 2009; Matheson and others 2012). Although there is strong evidence that light influences stream ecosystems at the reach scale, the light environment of natural streams is rarely uniform, particularly in headwater systems with riparian zones that contain forests with complex canopy structure (Keeton and others 2007; Warren and others 2013). Variability in the structure of the riparian forest canopy leads to variability in light along a stream and in turn to potential differences in the amount of primary production across the streambed (Carey and others 2007; Kiffney 2008; Stovall and others 2009). To date, however, few studies have explored when and how heterogeneous light environments affect variability in periphyton growth within a reach, or how patches of light may interplay with areas of nutrient limitation.

We examine the transitions between light and nutrient limitation of benthic algal communities along the length of a forested stream and address whether a heterogeneous light environment could cause stream benthic primary production to shift from light limitation to nutrient limitation at local scales, thereby creating reach-scale colimitation. We look across two different stand structures: a young forest with largely closed and uniform canopy structure and thus a relatively uniform light environment, and an old-growth forest with complex canopy structure yielding a more patchy stream light environment. We hypothesized that there would be a clear threshold at which periphyton accrual on the stream benthos transitioned from being predominantly light-limited to being predominantly nutrient-limited along a gradient of stream light exposure and that the two reaches would differ in the amount of light exposure above and below that threshold. A difference in the amount of nutrient versus light limitation associated with riparian forest age and structure could have important implications. The contrast in forest types evaluated here is representative of expected changes in midseral riparian forests over the next century as mature riparian forest transition to late succession conditions.

METHODS

Study Site

We conducted this study in McRae Creek, a third-order headwater stream on the western slope of the Oregon Cascades, located in the HJ Andrews Experimental Forest (HJA). Riparian forests in the HJA are dominated primarily by Douglas fir (*Pseudotsuga menziesii*), but in regenerating forests, red alder (*Alnus rubra*) is also an important component of the forest community. The study stream encompassed two stand development stages: early mature forest with dominant canopy trees between about 40–60 years of age, and old-growth forest with dominant canopy trees between about 400–500 years of age. The old-growth riparian canopy in this study was structurally complex with irregular canopy gaps that created local areas of increased light availability (Warren and others 2013). The riparian canopy of the midseral second-growth forest was more continuously closed with more hardwoods and relatively uniform structural composition, yielding lower and more consistent light availability along the stream in summer (Warren and others 2013). We established two separate light survey reaches each 160 m in length that were approximately 150 m apart (Figure 1). Although light exposure varied a great deal over short distances along the stream within our study reaches, background nutrient availability during the study was relatively consistent between the two reaches. Water samples were collected in early July 2015, just prior to project initiation that year. Three replicate water samples were collected at four locations (every 50 m) within each reach ($n = 8$). Samples were filtered on site (Whatman GF/F), frozen, and analyzed using a Dionex 1500 Ion Chromatograph (detection limit = $2 \mu\text{g L}^{-1}$ Nitrate-N). In the old-growth reach, nitrate (NO_3^- -N) concentration averaged $7.3 \mu\text{g L}^{-1}$ (SE $1.2 \mu\text{g L}^{-1}$) and phosphate (PO_4 -P) concentration averaged $8.6 \mu\text{g L}^{-1}$ (SE $0.6 \mu\text{g L}^{-1}$). In the second-growth reach nitrate (NO_3^- -N) concentration averaged $8.4 \mu\text{g L}^{-1}$ (SE $1.3 \mu\text{g L}^{-1}$) and phosphate (PO_4 -P) concentration averaged $7.9 \mu\text{g L}^{-1}$ (SE $0.5 \mu\text{g L}^{-1}$). Western Oregon's climate is characterized by mild year-round temperatures, relatively dry summers, and abundant winter precipitation. The entirety of this study was conducted during midsummer low-flow conditions during summer 2014 and 2015.

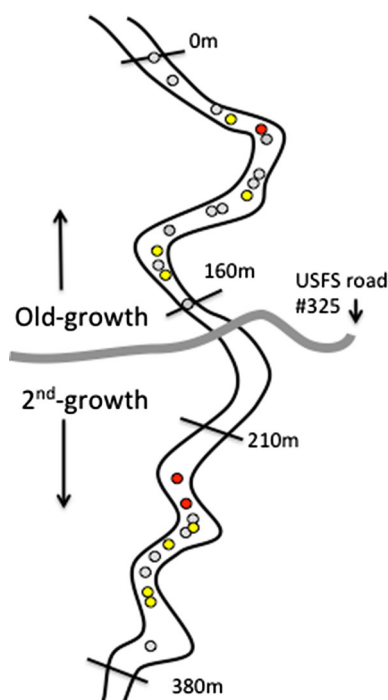


Figure 1. Approximate McRae creek NDS deployment locations. Study site schematic. Dot color indicates array type and study year: Light gray dots are 2014 deployment constrained NDS bioassays (including only control and a dual + nitrogen (N) and phosphorous (P) treatment), red dots are 2014 full bioassays (including control, +N, +P, and +N&P treatments) and yellow dots are 2015 constrained NDS bioassays locations (Color figure online).

Light Quantification

Light flux to the stream benthos was measured using two methods: fluorescein dye photodegradation and Odyssey light sensors that measured accumulated light exposure within the range of photosynthetically active radiation (PAR)—hereafter, “PAR meters” (Odyssey Photosynthetic Active Radiation Logger, Dataflow Systems, Christchurch, New Zealand) to record PAR. The fluorescein decay method relies on the predictable decay of fluorescein dye in sunlight to determine light exposure using methods applied in Bechtold and others (2012) and Warren and others (2013). Briefly, every five meters, we fastened three replicate 3.7 mL glass vials filled with buffered 400 ppb fluorescein to wire flags that were secured to the benthos in the center of the active channel of the stream. A total of 33 flags were deployed per reach. We were interested in quantifying the maximum potential light exposure on the stream benthos in these systems, therefore, fluorescein arrays were deployed for 24 h on days

with “full sun” (that is, <5% cloud cover) in midsummer. In the Mediterranean climate in which our study sites were located, rain is uncommon and most days are cloudless during the summer season. Weather during the study was typically dry and sunny and there were no rain events during the duration of the deployment in both years. Fluorescein arrays were deployed at sundown and retrieved at the same time the following day to capture one full day (24 h) of irradiance. To correct for any potential drift in the 400 ppb fluorescein standards, we deployed field-dark (foil-covered) vials every 20 m along with the three exposed vials. Upon retrieval, fluorescein bioassays were stored in the lab and kept in the dark until they all reached ambient room temperature, as sample temperature during measurement can affect fluorescence measurements (Bechtold and others 2012). Postdeployment fluorescein concentrations were measured using a Turner Designs AquaFluor handheld fluorometer to determine the overall change in fluorescein concentration in vials relative to the foil-covered controls in which there was no light exposure.

To translate the fluorescein photodegradation values to PAR, we developed a calibration curve using 20 fluorescein deployments (two periods of 10 deployments due to limited number of PAR meters). We secured the Odyssey light loggers just above the stream attached to rebar that was driven into the streambed within 0.1 m of where the fluorescein arrays were deployed. We then plotted fluorescein decay against the measured PAR at each site. We fit a two-factor polynomial relationship to the data setting the intercept to zero to ensure that no decay yielded a value of $0 \text{ mol m}^{-2} \text{ day}^{-1}$ of PAR. This yielded a relationship with a good fit ($r^2 = 0.85$; Online Appendix A). In nine of the periphyton accrual bioassays in 2015, PAR meters were deployed directly with the NDS bioassay substrates. For these sites, we used the 24-h-accumulated PAR values directly from the meters for the day on which the fluorescein bioassays were deployed.

Nutrient-Diffusing Substrates

To quantify nutrient limitation in McRae Creek, we deployed nutrient-diffusing substrate (NDS) bioassays across a range of light environments for 20 or 21 days over two summers (2014 and 2015) (Figure 1). Poly-con cup NDS's were prepared using the method similar to those detailed in Tank and others (2007). NDS bioassays comprised 2% agar amended with one of the four treatments: control

(unamended), nitrogen addition (1M N; NH_4Cl), phosphorous addition (1M P; KH_2PO_4), and nitrogen plus phosphorous additions (1M N; NH_4Cl and KH_2PO_4 P added at an N:P molar ratio of 1:1 per Capps and others (2011)). For the 2014 NDS bioassays, a glass fiber filter was placed on top of a fritted glass disc in each cup and the cups were all attached to a metal L-bar. In 2015, periphyton accrual was quantified directly on glass-fritted discs without glass fiber filters but all other sample processing steps were identical.

We made three full bioassays with control, +N, +P, and +N&P treatments with three replicates per treatment. These were deployed along with 15 condensed bioassays in summer 2014 (Figure 1). The condensed bioassays had only control and +N&P treatments. The condensed bioassays were used because the research question here focused on light versus nutrient limitation, and by deploying condensed NDS bioassays, we were able to nearly double the number of sites that we could survey. Evaluation of the +N&P only relative to control NDS was used to determine the presence or absence of nutrient limitation, but not which nutrient is limiting. By deploying more bioassays, we were able to evaluate a wider range of light conditions. We deployed the three full bioassays with the condensed bioassays along McRae Creek (Figure 1) but we made a point of putting the full sets in each of what we expected to be high, medium, and low-light locations based on a visual assessment of canopy cover during deployment. Nine condensed bioassays were deployed in summer 2015. All L-bars were placed in run or riffle habitats. We selected locations that had similar depth and water velocity based on visual assessments during deployment.

For each of the 27 total bioassays, after the 20 or 21-day stream incubation, glass filters or fritted discs were removed from each NDS bioassay for chlorophyll extraction and kept on ice until frozen. Filters/discs were kept frozen for 24 h, then thawed and chlorophyll α was extracted in 8 ml of 90% acetone at about 5°C. Samples were intermittently shaken throughout the incubation period. Chlorophyll α (hereafter Chl α) concentration in the resulting solution was determined by measuring fluorescence with a Turner Designs AquaFluor Model 8000-010 handheld fluorometer (Turner Designs, Sunnyvale, CA). After taking an initial reading, the solution was acidified with 0.1 molar HCl and allowed to stand for 90 seconds before fluorescence was measured again to correct for pheophytin interference (US EPA method 445). Measurement of Chl α concentration after the 20 or

21 day incubation was used as an indicator of overall periphyton accrual on the NDS. Although Chl α is a commonly applied proxy measure for periphyton accrual (for example, Capps and others 2011; Keck and Lepori 2012; Sanderson and others 2009; Tank and Dodds 2003), the relationship between periphyton Chl α and actual periphyton biomass can be affected by both light availability and nutrient concentrations (Rosemond 1993). In a preliminary study conducted in headwater streams in the McRae Creek basin in summer 2013—the year before we initiated the current project—we evaluated the relationships between Chl α and periphyton ash-free dry mass. We found a clear positive relationship between Chl α and AFDM looking across samples collected from 22 locations in headwaters of the McRae Creek stream network in the HJ Andrews Experimental Forest ($r^2 = 0.59$, $p < 0.001$; Online Appendix B). We also measured light at each of the stream periphyton sampling locations in the 2013 survey. We did not see a clear effect of light on the Chl α to AFDM ratio across these sites (Online Appendix B, $p = 0.719$, $r^2 = 0.007$). Based on these results and with the goal of increasing the number of bioassays that we could deploy and analyze in exploring a light versus nutrient limitation, we used only Chl α , and not AFDM as a measure of periphyton standing stocks on our NDS bioassays.

Light Limitation Versus Nutrient Limitation Threshold

Using a regression of mean Chl α accrual versus a 24-h-accumulated PAR, we evaluated the premise that mean Chl α accrual on NDS bioassays was related to light availability in this study system. The 24 h PAR data and the Chl α accrual data were log-transformed to achieve normality for the regression analysis. In exploring the transition from light limitation at sites with low light availability and nutrient limitation at sites with high light, we first calculated the mean and standard error of accrued Chl α concentration and compared control to nutrient-amended NDS for each L-bar location. We calculated both the difference in Chl α concentration between control and nutrient-amended NDS and the response ratio (control/nutrient-amended) between control and nutrient-amended NDS for each L-bar. Differences and response ratios were then plotted against the 24-h PAR value for a given L-bar location and a loess curve was fit to these relationships using the loess function in R (R Core Team 2014; <http://www.R-project.org>). We used the default smoothing parameter for the loess

function in R (value of 0.75). The loess curve had a clear inflection point, and the 24-h-accumulated PAR value at this inflection point was then used to determine the likely threshold at which the system transitioned from being primarily light-limited to being primarily nutrient-limited. The point of most rapid change (inflection point) was determined based on the maximum derivative of the loess fit (see Online Appendix C for derivative figures).

Combining the threshold at which the system transitions from being predominantly light to predominantly nutrient-limited with the longitudinal profile of stream light availability, we determined the proportion of the streambed over which periphyton growth was likely to be light versus nutrient-limited. We determined whether or not each of our high-resolution (every 5 m) stream light measurement points were above (nutrient-limited) or below (light-limited) a given threshold value (inflection point, and upper or lower 24-h PAR values determined from loess curve inflection point and residuals around the inflection point). Observed 24-h PAR values greater than the threshold were considered nutrient-limited, and values below the threshold were classified as light-limited. Then, the number of longitudinal survey locations above or below a given value was used to determine the proportion of each overall reach that are likely to be nutrient versus light-limited.

RESULTS

Stream Light

Light availability differed significantly between the two study reaches. The site with old-growth riparian forest had an average of about three times more light exposure (based on 24-h PAR) than the site with a midseral second-growth riparian forest (Table 1). Light fluxes in the old-growth reach were not uniformly greater, though. The old-growth reach had a number of locations where light fluxes were low and comparable to light fluxes in the second-growth reach, but the high-light areas in the old-growth reach were larger and more frequent than they were in the second-growth reach so maximum light values were higher (Figure 2). In addition to a greater total light flux, variability was also higher in the old-growth reach with a standard deviation of 24 h-accumulated PAR nearly double that of the midseral second-growth reach.

Nutrient-Diffusing Substrates

There were clear and significant relationships between 24 h PAR and Chl α accrual on both nutri-

Table 1. Stream Reach Lengths, Sampling Locations, Mean Light Availability, and % of the Reaches that are Light Versus Nutrient-Limited Using Threshold Transition and the End Members of the Range Over Which the System Shifts from Being Predominantly Light-Limited to Predominantly Nutrient-Limited

Reach	Reach length (m)	# PAR sampling locations	Mean 24 h PAR (mol m ⁻² day ⁻¹)	% streambed that is nutrient-limited based on 3.5 mol m ⁻² day ⁻¹ threshold (%)	% streambed that is nutrient-limited based on upper threshold (4.9 mol m ⁻² day ⁻¹) (%)	% streambed that is nutrient-limited based on lower threshold (2.2 mol m ⁻² day ⁻¹) (%)
Old-growth	160	33	8.0 (1.6)	73	58	76
Second-growth	160	33	2.8 (0.9)	27	9	30
Total	320	66	5.4 (0.6)	50	33	53

The 24-h-accumulated PAR estimates are derived from FL decay relationships. The upper and lower thresholds are based on NDS nutrient amendment responses across a range of natural light availability.

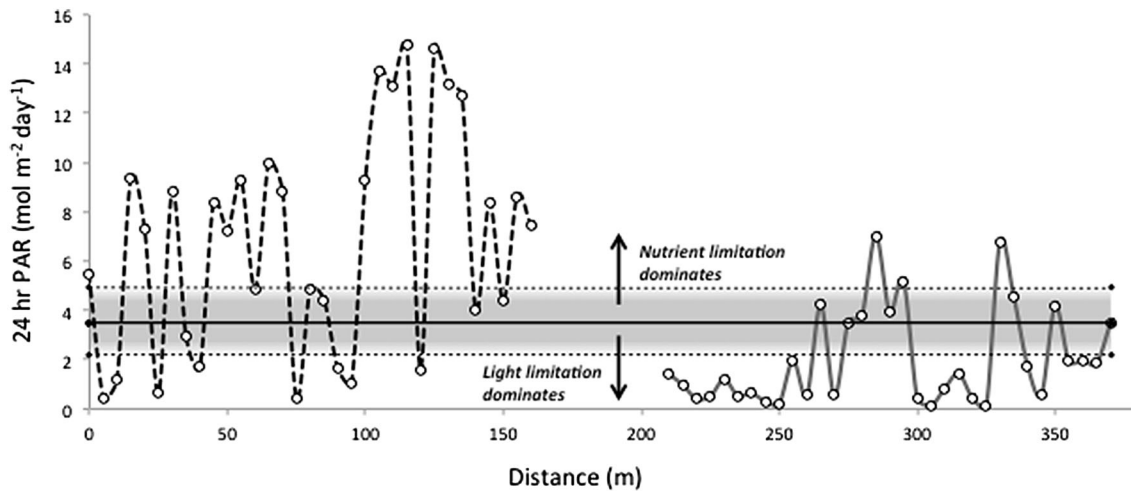


Figure 2. Twenty-four hour-accumulated PAR values in each study reach measured every 5 m with fluorescein dye decay methods. *Dashed line* (meters 0–160) indicates the reach with an old-growth riparian forest and the *solid line* (meters 210–370) indicates the reach with a second-growth riparian forest. *Solid straight line* indicates the threshold 24-h-accumulated PAR level at which the system shifts from light to nutrient limitation. When 24-h PAR values are above the *line*, benthic autotrophic production is primarily nutrient-limited and when 24 h PAR values are below the *line* benthic autotrophic production is primarily light-limited. *Dashed straight lines* and *gray box* indicate upper and lower error estimates on the threshold value based on residuals around the loess inflection point used to determine the transition threshold. Table 1 shows the % light versus nutrient-limited at either end of the range for each reach and for the combined stream section all together.

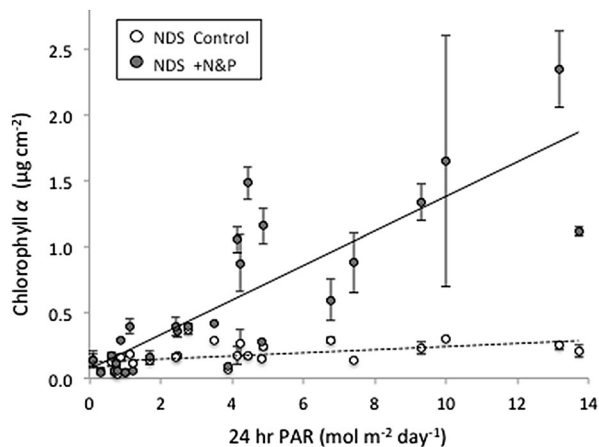


Figure 3. Periphyton chlorophyll *a* accretion on nutrient-diffusing substrate (NDS) bioassays deployed across a range of light availability in McRae Creek in summer 2015. *White dots* indicate Mean chlorophyll *a* on unamended NDS and *filled dots* indicate mean chlorophyll *a* on NDS amended with nitrogen and phosphorous together. *Error bars* represent 1 SE and are present on all data points but in many cases error is small and the *bars* are hidden behind the points themselves.

ent-amended and control NDS (linear regression, $p < 0.001$, $r^2 = 0.61$; and $p = 0.002$, $r^2 = 0.34$, respectively). However, the slope between light and Chl α is much greater for the nutrient-amen-

ded NDS than the control NDS (Figure 3). For the three full treatment bioassays (+N, +P, +N&P), the lowest light location ($0.4 \text{ mol m}^{-2} \text{ day}^{-1}$) had no significant differences among Chl α accretion on the control NDS and any of the nutrient-amended NDS suggesting no nutrient limitation. In the “midlight” location ($5.6 \text{ mol m}^{-2} \text{ day}^{-1}$), the +N treatment had an average of about 2.5 times more periphyton accretion than the control site and the +N&P treatment had an average of about 3.3 times more periphyton accretion suggesting N-limitation (Figure 4). Results from the larger assessment focusing on control versus +N&P treatments across a wide range of light fluxes further illustrated the influence of light on responses to the nutrient amendments. All four of the NDS bioassays in areas with 24-h PAR values less than $1.0 \text{ mol m}^{-2} \text{ day}^{-1}$ had no difference in Chl α accretion between nutrient-amended and control NDS (Figure 5A). At these low-light sites, the differences in Chl α accretion between control and nutrient-amended NDS were all less than 0.05 µg cm^{-2} . In contrast, the average difference for sites with PAR values greater than $4.9 \text{ mol m}^{-2} \text{ day}^{-1}$ was 0.94 µg cm^{-2} (SD = 0.53, $n = 11$; Figure 5A). The inflection point on the loess curve indicating a transition between light versus nutrient limitation in this reach occurred when 24-h PAR exceeded $3.5 \text{ mol m}^{-2} \text{ day}^{-1}$

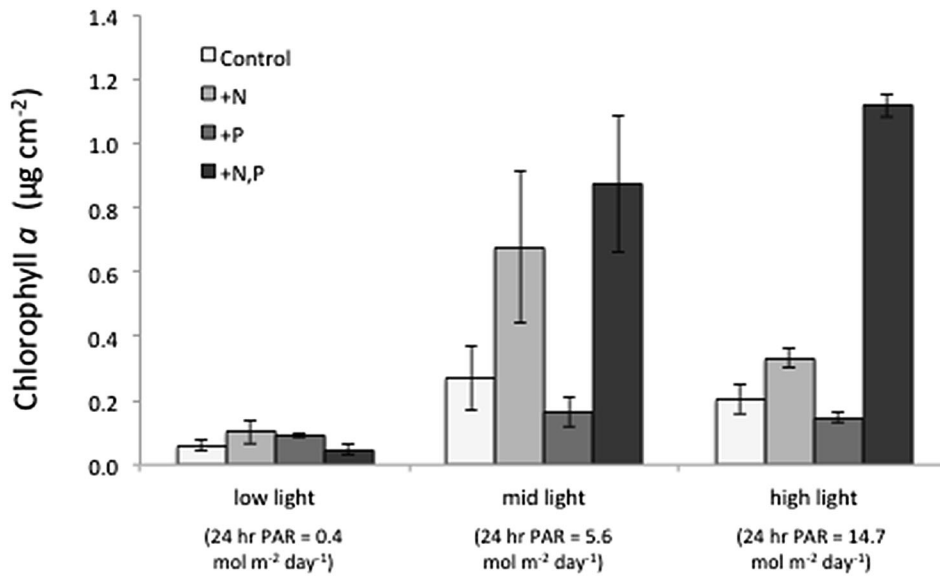


Figure 4. Results of NDS bioassays of accumulated Chl *a* with four treatments (Control, +N, +P, +N&P) across three light levels. Error bars represent one standard error.

(Figure 5A). The lower bound of the threshold was 2.2 mol m⁻² day⁻¹, and the upper bound was 4.9 mol m⁻² day⁻¹ (Figure 2; Online Appendix C).

Using the inflection point threshold, the old-growth reach had nearly three times more area that was nutrient-limited than the second-growth riparian forest reach (Table 1; Figure 2). Using lower and upper threshold values estimated from the derivative of the loess fit (Online Appendix C), the old-growth reach had between two and a half and six times more area that was nutrient-limited than the reach with second-growth forest (Table 1). Considering the whole stream section (combining both reaches), the inflection point threshold yielded a 50–50 split between light and nutrient limitation (Table 1; Figure 2).

DISCUSSION

Independent colimitation of benthic primary production in this system was an emergent property at the larger ecosystem scale that developed as a result of localized aggregate single-factor limitation (light *or* nutrients) in a heterogeneous light environment. Using nutrient-diffusing substrate bioassays to evaluate nutrient limitation versus light limitation of periphyton Chl *a* accrual, we found support for the hypothesis that there would be clear differentiation between the limiting factor for benthic primary production along a gradient of stream light exposure. Periphyton Chl *a* accrual was predominantly limited by nutrients (assessed with treatments of N&P together) in the high-light locations, and Chl *a* accrual was predominantly limited by light in low-light areas. We were able to identify a

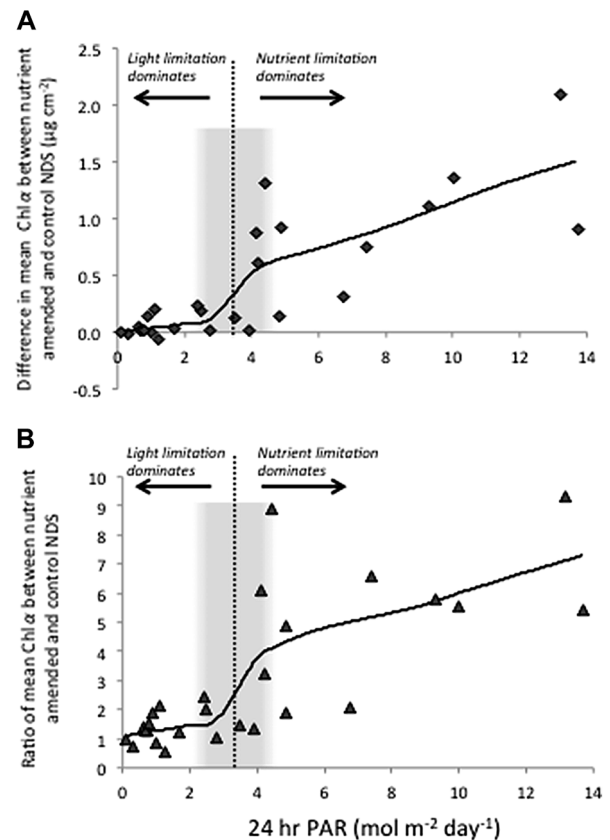


Figure 5. Differences (A) and ratios (B) of chlorophyll *a* on control versus nutrient-amended NDS across a range of light availability. Solid line is a loess fit to the data. Vertical dotted line is the inflection point of the fitted curve. This 24-h PAR value is used as the threshold for a transition from light to nutrient limitation. Gray box represents upper and lower ranges for the threshold estimated from the derivative of the loess fit (see Online Appendix C).

fairly constrained range of 24-h light exposure for our stream over which Chl α accrual transitioned between light and nutrient limitation. Although the specific light level at which a given system will transition between light and nutrient limitation will likely vary with background nutrient availability, and although background nutrient availability may influence photosaturation (Bothwell 1985; Hill and others 2011), this study clearly demonstrates that heterogeneous light availability can create or enhance reach-scale colimitation by light and nutrients in an oligotrophic system. Combining results from the threshold analysis with our high-resolution light measurements, we could identify areas of our study stream that were predominantly light-limited and others that were predominantly nutrient-limited.

Collectively, both study reaches were colimited at the larger ecosystem scale, however, the degree of light versus nutrient limitation varied between the reach with old-growth riparian forests and the reach with second-growth riparian forests. Differences in the proportion of sampling locations that were light versus nutrient-limited between the two study reaches suggest that stand development processes leading to greater canopy structural complexity can increase stream light and therefore increase nutrient limitation. The reach with old-growth forests had far more patches of high light and therefore a greater area over which the system was likely to be nutrient-limited relative to the reach with midseral second-growth forest. It is important to note that the results here do not preclude other forms of colimitation by light and nutrients (or by multiple nutrients). Indeed, in a set of well-constrained laboratory streams, Hill and others (2009) demonstrated through an experimental study that primary production could be strongly colimited by light and nutrients together, suggesting in this case that simultaneous colimitation and/or serial colimitation can also occur. Further, by using Chl α accumulated on NDS that were deployed for 21 days, we may be focusing on an incomplete periphyton community relative to the community that would develop on a natural substrate over time (Fisher and others 1982). Different communities can develop under different light and nutrient conditions (Hill and others 2011; Larned 2010; Stelzer and Lamberti 2001). And, the development of thick algal mats on a substrate can influence the level of light or nutrient saturation and maximum productivity (Bothwell 1989); however, we did not observe development of thick mats on our NDS bioassay over the duration of deployment in McRae Creek (nor did we observe

thick algal mats on the natural substrates in the study stream in summer 2014 or summer 2015). Despite their caveats in regard to community and biomass development, NDS bioassays are a well-established method for assessing nutrient limitation and given the absence of a light effect on Chl α :AFDM ratios in our 2013 preliminary study from the HJ Andrews, we are confident in the transition documented here between light limitation at low PAR and nutrient limitation at high PAR.

By influencing the timing and amount of primary production, stream light dynamics exert substantial controls on stream nutrient cycling. For example, Bernhardt and others (2003) found that in-stream processes could mitigate N export from a watershed by up to 50% following an ice storm that substantially reduced the amount of riparian vegetation. More broadly, GPP in streams has been shown to influence nitrogen (N) and phosphorous (P) retention on both seasonal and annual time scales (Bernot and others 2010; Finlay and others 2011; Julian and others 2011; Peterson and others 2001; Roberts and Mulholland 2007). At the reach scale, experimental increases in light availability along stream sections can create a biogeochemical hotspot at an intermediate scale (Collins and others 2016; Moslemi and others 2012), but to date, few studies have explored how small-scale light patches individually or in aggregate affect larger stream ecosystem processes. With the potential for stream autotrophs to not only increase production as a result of local increases in stream light below a canopy gap (Denicola and others 1992), but to also shift from being light-limited to nutrient-limited in the high-light areas below canopy gaps (Carey and others 2007), these areas may warrant particular consideration as important biogeochemical hotspots in streams as will the processes that create canopy gaps and other sources of spatial heterogeneity in stream light.

Although both stream reaches in this study would be characterized as “forested” in a general assessment of the system, the forest characteristics differed substantially, and these differences translated to over twice as much streambed area for which periphyton is likely to be nutrient-limited in the reach with old-growth riparian forests. Forest change has been and will continue to be an important influence on streams. Riparian forests are the dominant control on stream light in many temperate headwaters but forest canopies and canopy structure are not static. In North America, prior to European settlement, forested regions of North America were dominated by primary forests with complex, often old-growth, structures,

including variable tree density, frequent forest gaps, multiple canopy layers, and higher densities of large living and dead trees (Curzon and Keeton 2010; D'Amato and others 2009; Franklin and others 2002; Keeton and others 2007). Today, these regions are dominated by secondary forests recovering from earlier land clearing (Foster and others 1998; Pan and others 2011). These mostly young to mature forests tend to have less heterogeneous canopy structure both vertically and horizontally (Keeton and others 2007; Warren and others 2013; Van Pelt and Franklin 2000), which, in the current study translated to a dramatically different amount of the streambed that was light versus nutrient-limited. Studies have considered the interaction of stand development and stream ecosystem processes in regard to nutrient loading from the upland (Goodale and others 2000; Vitousek and Reiners 1975) as well as contributions of stream wood and its associated influences (Valett and others 2002; Warren and others 2007) but to date, few studies have considered how continued changes in riparian forest canopy structure will affect stream nutrient cycling via changes in autotrophic nutrient demand. Results from the current study highlight the importance of forest structure and they suggest that changes in canopy structure associated with stand development have the potential to dramatically change autotrophic nutrient demand in the streams.

Beyond the importance of GPP on stream nutrients, understanding factors that limit primary production and creating spatial variability in benthic primary production in streams provide insight into food-web dynamics and potential limits to secondary production within an ecosystem. Studies evaluating grazing and top-down controls on aquatic periphyton have long recognized the importance of small-scale spatial heterogeneity and periphyton community differences on stream primary production and food webs (Flecker and Townsend 1994; Townsend and others 1997; Winemiller and others 2010). Although leaf litter and other carbon inputs originating from outside the stream exceed in-stream production in most forested headwater streams, autochthonous carbon is on average higher quality food for consumers (that is, more nitrogen and phosphorous per unit carbon) (Cross and others 2005). Therefore algae and associated heterotrophic bacteria and fungi that grow on the stream benthos (collectively periphyton) comprise a key food resource at the base of stream food webs (even when present in low abundance), and in-stream primary production can strongly influence community structure de-

spite low relative standing stocks (Delong and Thorp 2006; Lau and others 2009a, b; McCutchan and Lewis 2002; Thorp and Delong 2002).

In considering the implications of these results beyond our specific stream, ecosystem scale colimitation is clearly a product of not only light variability but also background nutrient availability. Nutrient availability can vary spatially at local and landscape scales, but nutrient concentrations are also changing through time—due in large part to current or historic human activities—with streams in some areas experiencing increases in nutrients (Greene and others 2011; Vitousek and others 1997), whereas others experience declines (Renwick and others 2008). Increases in stream nutrient concentrations will enhance the importance of light as a driver of stream primary production, which will in turn increase the importance of riparian forest age, stage, and structure since riparian vegetation is the primary control on light in headwater streams.

Overall, this study provided empirical support for theoretical research discussing alternate pathways for colimitation at the ecosystem and community scale (Harpole and others 2011). An important driver of the independent colimitation in this study was spatial heterogeneity in resources that create conditions in which local single-factor (or predominantly single-factor) limitation of GPP could occur. Understanding how light variability along a stream influences and interacts with nutrient availability is important for understanding how stream ecosystems may change across North America in the coming century. Streams and streamside environments are dynamic. They change naturally through time and streams are often highly impacted both directly and indirectly by anthropogenic activities. Although anthropogenic changes to stream nutrients have received considerable attention in the literature, changes in stream light—which have also been profoundly impacted by human activities—have received less attention. These two factors are each important independently but their interaction and combined influence is also important and may warrant greater attention as we consider future changes in the structure and function of stream ecosystems.

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