

Resource synergy in stream periphyton communities

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Summary

1. Light and nutrients play pivotal roles in determining the growth of autotrophs, yet the potential for synergistic interactions between the two resources in algal communities is poorly understood, especially in stream ecosystems. In this study, light and phosphorus were manipulated in large experimental streams to examine resource colimitation and synergy in stream periphyton.

2. Whole-stream metabolism was simultaneously limited by light and phosphorus. Increasing the supply of either light or phosphorus resulted in significant increases in primary production and the transformation of the streams from heterotrophy to autotrophy.

3. Resource-driven changes in periphyton community structure occurred in concert with changes in production. Algal assemblages in highly shaded streams were composed primarily of small diatoms such as *Achnantheidium minutissima*, whereas larger diatoms such as *Melosira varians* predominated at higher irradiances. Phosphorus enrichment had relatively little effect on assemblage structure, but it did substantially diminish the abundance of *Meridion circulare*, a diatom whose mucilaginous colonies were conspicuously abundant in phosphorus-poor, high-light streams. Bacterial biomass declined relative to algal biomass with increases in primary productivity, regardless of whether the increases were caused by light or phosphorus.

4. Synergistic effects on primary production appeared to occur because the availability of one resource facilitated the utilization of the other. Light increased the abundance of large diatoms, which are known to convert high concentrations of nutrients into primary production more effectively than smaller taxa. Phosphorus enrichment led to the replacement of *Meridion circulare* by non-mucilaginous taxa in phosphorus-enriched streams, and we hypothesize that this change enabled more efficient use of light in photosynthesis. Higher ratios of chlorophyll *a* : biomass in phosphorus-enriched streams may have also led to more efficient photon capture and higher photosynthetic rates.

5. *Synthesis.* Our results underscore the potential for resource colimitation, even in habitats where a single resource is as strongly limiting as is light in shaded streams. The capacity of autotrophic communities to respond to more than one limiting resource suggests that prevailing single-resource models of ecosystem productivity are overly simplistic.

Key-words: Algae, aquatic plant ecology, bacteria, community structure, light, phosphorus, primary production, resource synergy, streams

Introduction

Supplies of light and nutrients are fundamental determinants of community metabolism in aquatic ecosystems. When these autotroph-essential resources are scarce, endogenous primary production is suppressed and the heterotrophic utilization of terrestrially derived organic matter rises in importance (Duarte & Agusti 1998; Cotner & Biddanda 2002). The attenuation of solar radiation by riparian vegetation and suspended inorganic

particles commonly limits *in situ* photosynthesis in fluvial ecosystems and, even when photons are abundant, low-nutrient concentrations can constrain autotrophy in these ecosystems (Peterson *et al.* 1985; Mulholland *et al.* 2001; Battin *et al.* 2008). In the pelagic zones of oligotrophic lakes and oceans, phytoplankton photosynthesis is frequently outweighed by bacterial catabolism of terrestrially derived dissolved organic matter (DOM; del Giorgio, Cole & Cimleris 1997; Ask *et al.* 2009; Sand-Jensen & Staehr 2009); increasing the supply of nutrients to these zones stimulates the production of large algae, increases the ratio of algal to bacterial biomass and

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raises net community primary production to positive values (Duarte *et al.* 2000; Biddanda, Ogdahl & Cotner 2001).

Although light and nutrients have been widely investigated as individual resources, less attention has been directed towards their combined effects on aquatic primary productivity. Single-factor models of ecosystem productivity have tended to dominate discussions of resource limitation. These models neither account for the heterogeneity of multiple resource use in natural ecosystems nor do they address potential synergies between resources (e.g. Davidson & Howarth 2007). The failure of the nutrient limitation paradigm (widely promulgated by limnologists) to explain strong effects of light on the production of autotrophs, invertebrates and fish in nutrient-poor lakes exemplifies the shortcomings of single-resource models (Karlsson *et al.* 2009). Because light and nutrients are often simultaneously scarce in aquatic habitats, the potential for these two resources to colimit primary production could be widespread.

Several mechanisms have been proposed that could account for colimitation and resource synergy, although the extent and efficacy of these mechanisms are poorly understood (Davidson & Howarth 2007). At the level of a single algal cell, colimitation can occur when the availability of one resource influences the cell's acquisition and use of another resource. Nitrogen availability is known to affect the ability of algae to photoacclimate to low irradiances, enabling the synthesis of additional photosynthetic pigments and membranes that increase the efficiency by which cells capture sparse photons (Prezelin & Matlick 1983). Phosphorus may enhance algal photoacclimation as well (Hessen, Faerovig & Anderson 2002), although experimental support for the effects of phosphorus enrichment at low irradiances is limited (Hill & Fanta 2008). At the community level, heterogeneity in resource distribution may engender colimitation because the availability of limiting resources can differ considerably from one part of the community to the other (e.g. Hautier, Niklaus & Hector 2009). Heterogeneity of utilization may also create the potential for colimitation, as different species are likely to have different requirements for resources (e.g. Litchman & Klausmeier 2008). Danger *et al.* (2009) theorize that colimitation is common in multispecies assemblages because selection pressure encourages the establishment of dominant species with resource requirements matching the relative supplies of different resources.

The light : nutrient hypothesis argues that light and nutrients interact antagonistically rather than synergistically (Sterner *et al.* 1997). Contrasting effects of light and nutrients on algal nutrient content are postulated to have far-reaching consequences for multiple ecosystem processes, including bacterial production. The relationship between bacteria and algae is predicted to be sensitive to light : nutrient ratios because bacterial growth is putatively dependent on algal exudates. Algae in oligotrophic waters are hypothesized to excrete progressively more DOM as light : nutrient ratios increase because nutrient uptake is unable to keep pace with carbon fixation and excess carbon is released as DOM (Sterner *et al.* 1997). The link between

bacterial growth and the availability of DOM leads to the prediction that bacterial growth rates and the ratio of bacterial biomass to algal biomass will increase as light : nutrient ratios rise (Elser *et al.* 2003). It is not clear how well this planktonic model of bacterial–algal interactions applies to stream periphyton communities, however. Bacteria in periphyton communities rely on attached algae for habitat structure as well as for nutrition, so bacterial biomass may be more closely tied to algal biomass in these communities than in open-water habitats. Positive responses by algae to light enrichment may also overwhelm any DOM-linked increase in bacterial production, regardless of habitat. Predictions based on the light : nutrient hypothesis presuppose that bacterial reliance on terrestrially derived organic matter is relatively minor.

In this study, we investigated the potential for light and phosphorus to act synergistically on the balance of autotrophy and heterotrophy in stream periphyton. We tested the capacity of one resource to compensate for scarcity in the other, causing increases in autotrophic production not predicted by single-resource models. Stream-bed irradiances and dissolved phosphorus were simultaneously manipulated in large experimental streams that were amenable to whole-stream measurements of primary production and respiration. Previous work by our group explored the combined effects of light and phosphorus on periphyton stoichiometry and growth (Hill & Fanta 2008; Hill, Fanta & Roberts 2009; Fanta *et al.* 2010); this study focused on stream metabolism and the connections between metabolism and community structure. We hypothesized that primary production would be colimited by light and phosphorus at subsaturating resource levels and that augmenting the supply of one resource would enhance the utilization of the other. We examined the consequences of resource enrichment on the community structure in the streams, looking for changes in species composition that could be linked to synergistic effects on primary production. We also explored the effects of resource enrichment on the relationship between bacteria and algae, testing the light : nutrient hypothesis that bacterial populations are favoured by high light : nutrient ratios.

Methods and materials

EXPERIMENTAL STREAMS

Six flow-through streams at the Oak Ridge National Laboratory indoor stream facility were used in this study. Each stream was 22 m long by 0.3 m wide and supplied with unfiltered, low-nutrient water from nearby First Creek. A flow rate of 0.3 L s⁻¹ was maintained in each stream by adjusting an inflow valve at the head of the stream. At this flow rate, bulk water velocity is *c.* 10 cm s⁻¹ and the hydraulic residence time is 3.7 min in the streams (Hill, Fanta & Roberts 2009). Stream substrata were continuous mats of unglazed, 2.4 cm × 2.4 cm white ceramic tiles that were readily colonized by algae and bacteria from First Creek. Light was provided on a 14 : 10 hour light : dark cycle with seven 400-W metal halide lamps hung from the ceiling above each stream. These lamps provided streambed irradiances of *c.* 110 μmol photons m⁻² s⁻¹ in unshaded streams.

EXPERIMENTAL DESIGN

Three levels of light and two levels of phosphorus were applied on a whole-stream basis in a factorial design, with each of the six streams having a unique combination of light and phosphorus. The three light levels were established by placing 0, 1 or 2 layers of plastic window screening over the streams. Each layer of screening reduced light penetration by *c.* 60% and resulted in streambed irradiances that were close to 110, 40 and 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The highest irradiance was expected to be growth-saturating whereas the lowest irradiance was expected to be severely growth-limiting (Hill, Fanta & Roberts 2009). The two phosphorus levels were established by enriching half of the streams with phosphorus and leaving the other half unenriched. Phosphorus enrichment was achieved by pumping a concentrated stock solution of Na_2PO_4 from carboys at the heads of the three enriched streams with peristaltic pumps at rates calculated to achieve in-stream concentrations of *c.* 75 $\mu\text{g P L}^{-1}$. The phosphorus concentration in the other three streams was that of incoming First Creek water, initially estimated to be 5 $\mu\text{g L}^{-1}$. Phosphorus concentrations of 5 and 75 $\mu\text{g P L}^{-1}$ were expected to be growth-limiting and growth-saturating, respectively (Hill, Fanta & Roberts 2009). To eliminate the potential for nitrogen limitation (background concentrations were *c.* 100 $\mu\text{g N L}^{-1}$), a concentrated solution of NaNO_3 was pumped into all streams at a rate calculated to achieve in-stream concentrations of *c.* 400 $\mu\text{g N L}^{-1}$.

The treatments were replicated in time by performing two sequential experiments: experiment 1 was performed 25 November to 13 December 2006, and experiment 2 was performed 15 December 2006 to 3 January 2007. The specific treatment combination assigned to each stream was randomized for each experiment. Stream substrata were vigorously brushed between experiments to remove periphyton and minimize carryover effects.

SAMPLING

Detailed measurements of discharge, temperature, nutrients and light were made in all streams to ensure treatment fidelity and quantify experimental variability. Discharge was measured daily and adjusted as needed to conform to 0.3 L s^{-1} . The output of the peristaltic pumps was also checked daily and adjusted as needed to ensure constant enrichment. Stream temperature at the downstream end of each stream was monitored daily with a calibrated digital thermometer. Water samples for soluble reactive phosphorus (SRP) and nitrate were taken every 2 days at the midway point (11 m) of each stream; the samples were filtered through glass-fibre filters (Whatman GFF, Whatman Inc., Piscataway, NJ, USA) at the time of sampling and frozen within 30 min for subsequent analysis. Photosynthetically active radiation was measured at the beginning of each experiment with a quantum sensor (LiCor 190SA) placed at 46 locations encompassing the length of each stream.

Periphyton was sampled on the last day of both experiments (13 December, 3 January) and on 2 additional days in the second experiment (22 December, 28 December). One periphyton-covered tile was carefully removed from the stream at each of three locations (8, 14 and 19 m downstream of the head of each stream) and placed in an ice-filled cooler until all the streams had been sampled. Periphyton was removed from the tiles by brushing with a stencil brush and rinsing with filtered stream water. The periphyton brushed from the three tiles collected from each stream was combined into a single sample per stream. Aliquots from the periphyton slurry were filtered onto pre-ashed, pre-weighed glass-fibre filters (Whatman GFF) and stored at -80°C for later analysis of chlorophyll *a* (Chl *a*) and ash-free dry mass

(AFDM). Aliquots for algal and bacterial biovolume analysis were also taken from the slurry; algal samples were preserved with Lugol's solution and bacterial samples were preserved with glutaraldehyde.

SAMPLE ANALYSIS

Water samples were analysed for SRP using the ascorbic acid method and a 5-cm spectrophotometric pathlength (APHA 2005). Nitrate was analysed by the second-derivative spectrophotometric method of Crumpton *et al.* (1992). Periphyton AFDM was determined by drying periphyton filters at 60°C , weighing the filters, exposing them to 500°C and then reweighing the filters.

Filters for Chl *a* analysis were immersed in 90% ethanol in centrifuge tubes, sonicated with a Branson probe sonicator for 30 s at 50 W, and then allowed to extract in the dark for 24 h. The ethanol was maintained at 4°C during all parts of the extraction process to minimize pigment degradation. The extract was clarified by centrifugation at 4°C and analysed spectrophotometrically for Chl *a* concentration, using its specific absorption coefficient (11.99) (Sartory & Grobbelaar 1984). Chl *a* was corrected for pheopigments. Filters for AFDM analysis were dried at 60°C , weighed, placed in a 480°C oven for 2 h and then weighed again.

Algal biovolume was determined by examining preserved samples with an inverted microscope at 1000 \times . At least 500 cells were enumerated in each sample. Diatoms were identified to species level with the aid of subsamples cleared with hot H_2O_2 and mounted in Naphrax (Northern Biological Supplies Ltd, Ipswich, UK). Soft algae were identified to genus level. Cell volumes were calculated with standard geometric formulae using dimensions measured on at least 10 cells per taxon.

Bacterial biovolume was determined by epifluorescence microscopy and digital image analysis. Sample aliquots were filtered onto aluminium oxide discs (Anodisc; Whatman, 25 mm, 0.2 μm pore size), stained with a drop of 2.5% SYBER Gold I (Molecular Probes, Inc.) and mounted in anti-fade solution (50% Glycerol, 50% PBS, 0.1% *p*-phenylenediamine). At least 10 random fields (≥ 300 cells) of each sample were examined at 1000 \times . Bacteria were enumerated and categorized into shape/size classes. Mean cell volume of each shape/size class was determined by capturing size-calibrated digital images and employing the image filters and biovolume calculations of Masana *et al.* (1997). With the exception of one rare shape/size class, at least 30 cells were measured for each class. CMEIAS Ver. 1.27 (Liu *et al.* 2001) operating in the UTHSCSA Image Tool Ver. 1.27 was used to determine to cell areas and perimeters from digital images.

WHOLE-STREAM METABOLISM

Ecosystem metabolic rates were measured using a two-station, open-system method. Measurements of dissolved oxygen (DO) and water temperature were made at 4-min intervals over 24-h periods at the upstream and downstream ends of the streams with YSI model 600XL sondes equipped with model 6952 DO probes (YSI Inc., Yellow Springs, OH, USA). DO per cent saturation was determined from DO concentration, water temperature and barometric pressure (measured at a site in nearby Walker Branch [Roberts, Mulholland & Hill 2007] and corrected for the elevation difference between the sites by comparing > 50 measurements).

Volumetric ecosystem metabolism rates ($\text{g O}_2 \text{m}^{-3}$) were determined from the rate of change in DO concentration using the equation $\Delta\text{DO} = \text{GPP} - \text{CR} + E$, where ΔDO is the change in DO concentration, GPP is the gross primary production ($\text{g O}_2 \text{m}^{-3}$), CR is the community respiration, and E is the net exchange of O_2 with the

atmosphere between the upstream and downstream sondes. E is the product of the O_2 reaeration coefficient (k_{O_2}) and the average DO deficit (DO concentration at 100% saturation minus the DO concentration in stream water) over the measurement interval. Reaeration coefficients were determined using simultaneous, continuous injections for propane gas (volatile tracer) and a concentrated NaCl solution (conservative tracer) in several streams on multiple dates during each experiment (total of nine injections) following the methods detailed in Roberts, Mulholland & Hill (2007). The O_2 reaeration coefficient varied with mass of periphyton in the streams and was therefore calculated according to the equation $k_{O_2} = 0.0399 \times \ln(g \text{ AFDM}) + 0.1079$ ($r^2 = 0.95$, $P < 0.0001$). Daytime CR was determined by interpolating CR averaged over the hour before dawn and the first hour after dusk (Roberts, Mulholland & Hill 2007). GPP for each daytime interval was the sum of the net metabolism flux and interpolated CR. Daily volumetric GPP and CR rates ($g \text{ O}_2 \text{ m}^{-3} \text{ day}^{-1}$) were calculated as the sum of the 4-min rates over each 24-h period. Volumetric rates were converted to areal units ($g \text{ O}_2 \text{ m}^{-2} \text{ day}^{-1}$) by dividing by the mean water depth (determined from the NaCl injections; Roberts, Mulholland & Hill 2007) of each stream. As with k_{O_2} , mean depth (Z_{mean}) varied with periphyton AFDM, and was therefore calculated for each stream and day according to the equation $Z_{\text{mean}} = 0.0027 \times \text{AFDM} + 0.0115$ ($r^2 = 0.85$, $P < 0.001$). Only the metabolism data from days on which periphyton was sampled are reported in this study.

DATA ANALYSIS

Light and phosphorus effects were tested with blocked, two-way analysis of variance (ANOVA) in which the two experiments were treated as blocks. Data were log-transformed or arcsine-transformed (relative biovolumes) before ANOVA to satisfy the assumption of homoscedasticity. Because of differences between the two experiments in algal assemblage composition, ANOVA was used to test treatment effects only on four taxa that were major contributors to algal biovolume in both experiments. The whole-stream application of treatments limited our ability to replicate treatments ($n = 2$ for each Light \times Phosphorus combination) and constrained experimental power, so critical F -values were not Bonferroni-adjusted. Spearman's nonparametric correlation analysis (ρ) was used to test associations between variables that appeared to be nonlinearly related.

Non-metric multidimensional scaling analysis (NMDS) was performed to compare the algal assemblages that developed in the different experiments and streams. Relative biovolume data were arcsine, square-root transformed before being used to construct Bray–Curtis similarity matrices that were then used in NMDS analysis. Primer© version 6.1.6 was employed for the analysis.

Results

EXPERIMENTAL CONDITIONS

Light and phosphorus treatments were close to targeted levels and consistent from one experiment to the other. Mean irradiances for the low, medium and high light treatments in the two experiments were 17 ± 0.4 (SE), 39 ± 0.6 and $111 \pm 3 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively ($n = 4$). Irradiances in individual streams varied by $< 8\%$ within treatments. Mean SRP concentrations for the low and high phosphorus treatments were 4 ± 0.2 and $82 \pm 11 \mu\text{g P L}^{-1}$, respectively ($n = 6$). Nitrate

concentrations were steady over time and averaged $390 \pm 75 \mu\text{g N L}^{-1}$ ($n = 12$). Stream temperature varied on a daily basis depending on outside weather conditions, ranging from 9 to 15 °C in the first experiment and 11 to 13 °C in the second. Mean stream temperature in both experiments was 12 °C. Individual streams differed by ≤ 0.4 °C.

PERIPHYTON BIOMASS

Periphyton accrual in the experimental streams was significantly affected by both light and phosphorus (Fig. 1, Table 1). Phosphorus-enrichment magnified light effects on Chl a accrual, resulting in quantities that were two times greater in phosphorus-enriched, high-light streams than they were in phosphorus-poor, high-light streams (Fig. 1a). AFDM accrual was more strongly influenced by light than phosphorus, although phosphorus enrichment did result in AFDM levels 30–40% higher at the highest light treatment (Fig. 1b). The

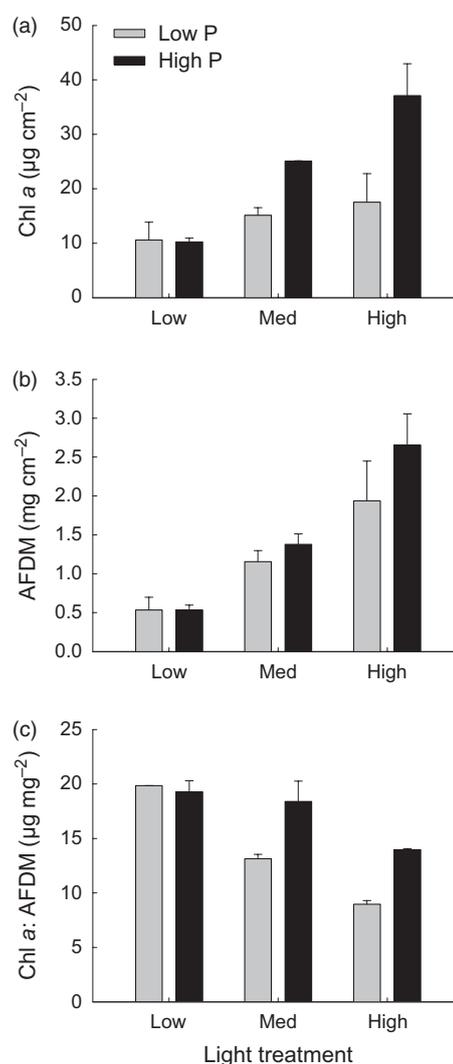


Fig. 1. (a) Periphyton chlorophyll a (Chl a), (b) ash-free dry mass (AFDM) and (c) Chl a : AFDM in the experimental streams. Each symbol represents the mean ± 1 standard error of data from two streams.

Table 1. Analysis of variance results

Parameter	Transformation	Experiment ($F_{1,5}$)	Light ($F_{2,5}$)	Phosphorus ($F_{1,5}$)
Chlorophyll <i>a</i>	Log	8.7*	25**	16**
Ash-free dry mass	Log	24**	129***	6.3*
Chl <i>a</i> : AFDM	Log	1.3	64***	37**
Gross primary production	Log	2.0	150***	25**
Respiration	Log	6.2	391**	109***
Net community production	Log	0.3	177***	18**
<i>Achnanthydium minutissima</i> %	Arcsine	3.0	9.2*	0.1
<i>Gomphonema angustatum</i> %	Arcsine	3.8	16**	0.1
<i>Melosira varians</i> %	Arcsine	14*	7.4*	0.7
<i>Meridion circulare</i> %	Arcsine	2.8	1.2	22**

F-values for the effects of experiment, light and phosphorus on periphyton biomass, metabolism and relative biovolumes of four algal species common to both experiments. All data analysed came from the last day of each experiment. Light \times Phosphorus interaction terms are not included because they were not significant ($P > 0.05$) after data transformation.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ratio of Chl *a* to AFDM decreased *c.* 50% at the highest light level in phosphorus-poor streams whereas it decreased only *c.* 25% at the highest light level in phosphorus-enriched streams (Fig. 1c).

ALGAL ASSEMBLAGE STRUCTURE

Diatoms comprised 23 of the 25 taxa identified in both experiments and accounted for >95% of total algal biovolume. Despite the overall dominance by diatoms, assemblage composition did differ between experiments and between experimental treatments. NMDS of relative biovolumes illustrated these differences (Fig. 2). The first NMDS axis separated assemblages primarily by experiment, whereas the second axis separated assemblages by light and phosphorus treatments. Assemblages growing under the lowest irradiances clearly differed from assemblages growing under the two higher irradiances in both experiments. Assemblages from the two higher irradiances were not separated by light treatment, but they

were separated according to phosphorus treatment. Within each light treatment, phosphorus enrichment consistently reduced second axis scores. The reduction was smaller at the lowest light treatment.

Differences between experiments in assemblage structure were due primarily to the overwhelming dominance of *Melosira varians* at medium and high irradiances in the second experiment (Fig. 3). This chain-forming diatom was only co-dominant in the first experiment. In both experiments, small species such as *Achnanthydium minutissima* and *Gomphonema angustatum* contributed greatly to algal biovolume at low light levels, whereas larger species such as *Synedra acus* and *Melosira varians* were more important at higher light levels. Mean cell volume (μm^3) was highly correlated with streambed irradiance ($\rho = 0.70$, $P = 0.01$, $n = 12$). It is clear from Fig. 3 that algal assemblage structure was affected more by light than by phosphorus. ANOVA results demonstrated significant effects of light alone on the relative biovolumes of *A. minutissima*, *G. angustatum* and *Melosira varians* (Table 1).

Meridion circulare was highly sensitive to phosphorus enrichment, in contrast to the other algal taxa. This colonial diatom was much more abundant in phosphorus-poor streams, both in terms of % biovolume (Fig. 3) and in terms of absolute (areal-specific) biovolume. The mean areal-specific biovolume of *Meridion circulare* in the phosphorus-poor streams was at least twice that in phosphorus-enriched streams: 0.09 vs. 0.04 $\text{mm}^3 \text{cm}^{-2}$ (low irradiance), 0.45 vs. 0.20 $\text{mm}^3 \text{cm}^{-2}$ (medium irradiance) and 1.08 vs. 0.54 $\text{mm}^3 \text{cm}^{-2}$ (high irradiance). Light and phosphorus effects on areal-specific biovolume were both highly significant ($P < 0.01$), as were phosphorus effects on relative biovolume (Table 1). The gelatinous colonies of *Meridion circulare* were visually conspicuous in high-light, phosphorus-poor streams.

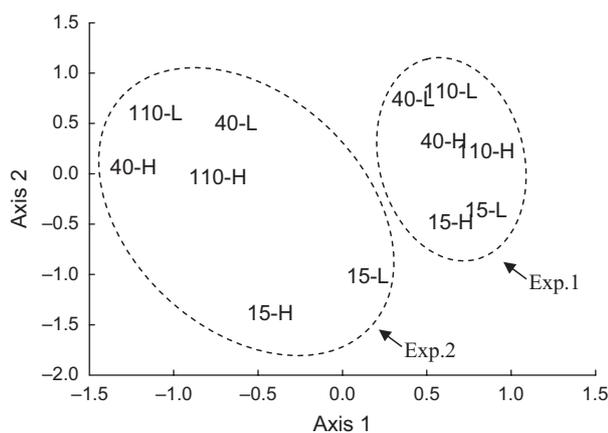


Fig. 2. Non-metric multidimensional scaling analysis (NMDS) of algal community composition. Each symbol represents a single stream. The numbers in the symbols are the nominal irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for the stream and the letters are the phosphorus level in the stream ('L' = low, 'H' = high). NMDS stress value = 0.07.

BACTERIAL AND ALGAL BIOVOLUME

Algal biovolume was highly correlated to periphyton AFDM in both experiments (Fig. 4a,b), as expected. Bacterial biovolume was also positively correlated with AFDM, although the relationship was statistically significant only in the second

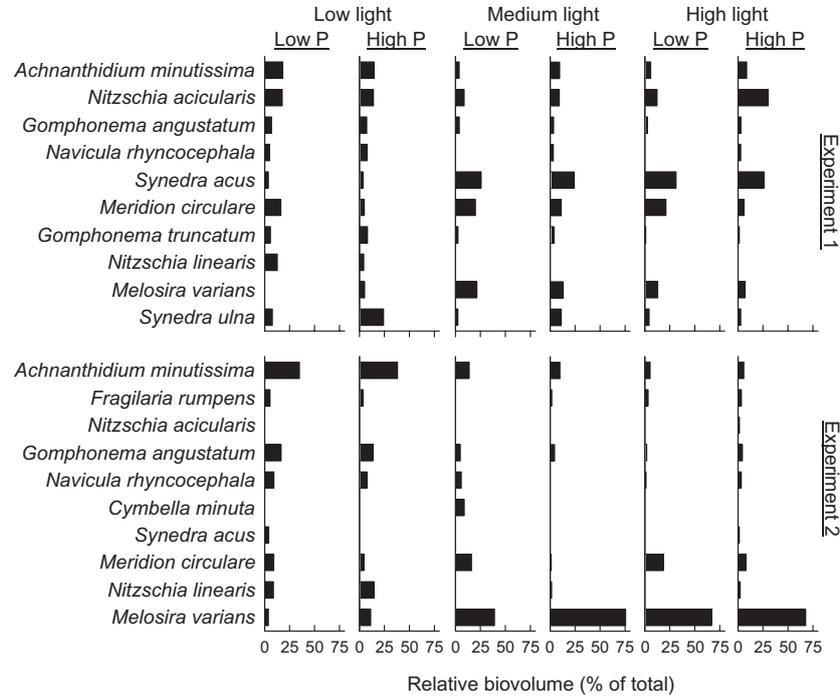


Fig. 3. Relative biovolumes (% of total biovolume) of individual algal taxa in the experimental streams. The 10 species contributing the most to total algal biovolume are shown; these species constituted > 88% of algal biovolume in each stream. Species are ordered (top to bottom) by size (smallest to largest). Each panel box represents an individual stream.

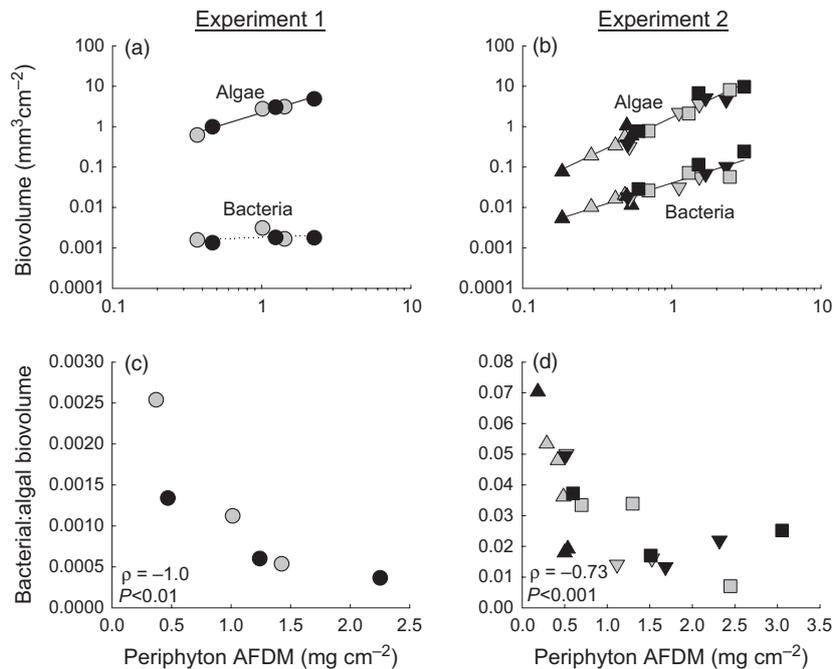


Fig. 4. Algal and bacterial biovolume. (a) Biovolume vs. periphyton biomass in experiment 1. Regression equations are: \log algal biovolume = $0.34 + 1.13 \times \log$ AFDM ($r^2 = 0.97$, $P < 0.001$); \log bacterial biovolume = $-2.74 + 0.14 \times \log$ AFDM ($r^2 = 0.11$, $P = 0.51$). (b) Biovolume vs. periphyton biomass in experiment 2. Regression equations are: \log algal biovolume = $0.23 + 1.74 \times \log$ AFDM ($r^2 = 0.95$, $P < 0.001$); \log bacterial biovolume = $-1.40 + 1.17 \times \log$ AFDM ($r^2 = 0.89$, $P < 0.001$). (c) Bacterial biovolume : algal biovolume vs. AFDM in experiment 1; Spearman's ρ is shown. (d) Bacterial biovolume : algal biovolume vs. AFDM in experiment 2; Spearman's ρ is shown. Black and grey symbols represent high and low phosphorus, respectively. Circles, triangles, inverted triangles and squares represent samples taken on 13 December, 22 December, 30 December and 3 January, respectively.

experiment. The increase in bacterial biovolume with increasing AFDM was less than that of algal biovolume, so the ratio of bacterial biovolume to algal biovolume decreased as AFDM increased in both experiments (Fig. 4c,d).

STREAM METABOLISM

Whole-stream metabolism was simultaneously limited by light and phosphorus. Light augmentation increased GPP fivefold or more in both phosphorus-poor and phosphorus-enriched streams, and the effects of phosphorus enrichment were apparent even at the lowest irradiances, which were obviously well below photosaturation (Fig. 5a). CR also increased with increasing light and phosphorus (Fig. 5b), but the increase was milder than that of GPP. Consequently, net community production (NCP) responded markedly to increasing resource supply (Fig. 5c). In the streams with the lowest light levels, CR exceeded GPP, and NCP values were negative (heterotrophic).

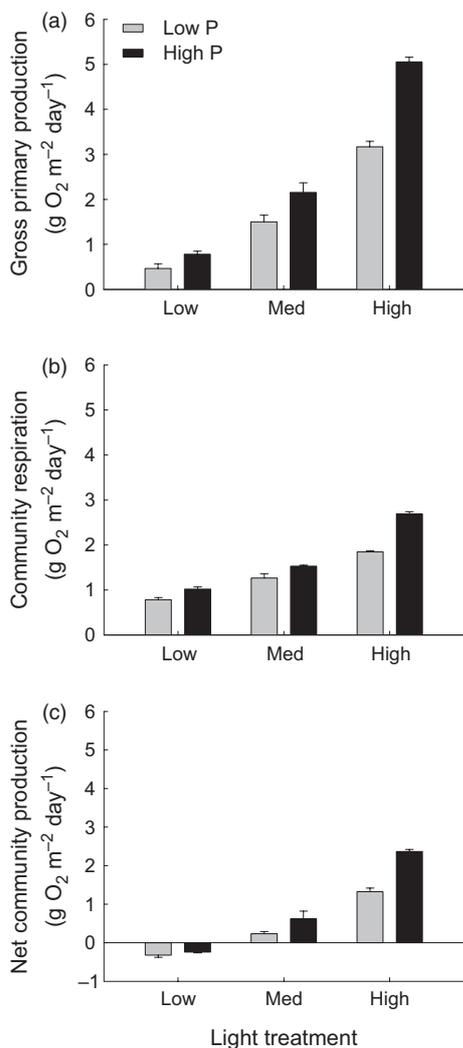


Fig. 5. (a) Gross primary production, (b) community respiration and (c) net community production as a function of light and phosphorus concentration. Each bar represents the mean \pm 1 standard error of metabolism in two streams.

Light and phosphorus effects on log-transformed metabolic rates were highly significant in the ANOVA (Table 1). Statistical analysis of untransformed GPP, CR and NCP data resulted in highly significant Light \times Phosphorus interaction terms, but the terms became statistically insignificant ($P > 0.05$) when the data were transformed to satisfy assumptions of variance homogeneity.

Whole-stream GPP, CR and NCP were highly correlated with the mean cell size (volume) of algal cells in the streams (Fig. 6). All three stream metabolism measures were highly correlated with periphyton AFDM ($\rho > 0.92$, $P < 0.001$).

Discussion

Light and phosphorus acted synergistically in transforming the experimental streams from heterotrophy to autotrophy. Augmenting the supply of either resource resulted in rapidly increasing primary production throughout the range of treatment combinations, raising the net daily metabolism from negative to positive. Colimitation was apparent even at low resource levels: light augmentation greatly stimulated

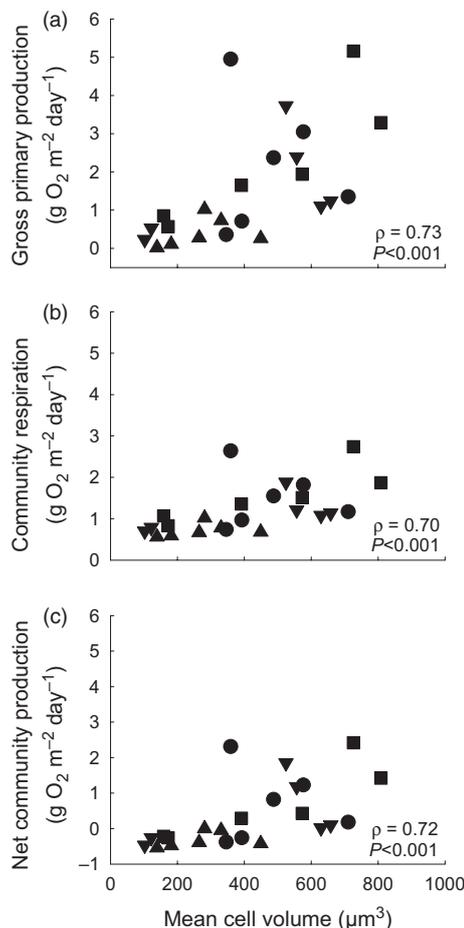


Fig. 6. (a) Gross primary production, (b) community respiration and (c) net community production vs. algal cell size. Circles, triangles, inverted triangles and squares represent whole-stream metabolism on 13 December, 22 December, 30 December and 3 January, respectively.

GPP and NCP in phosphorus-deficient streams despite SRP concentrations that were far less than growth-saturating ($c. 25 \mu\text{g L}^{-1}$; Hill, Fanta & Roberts 2009), and phosphorus enhanced GPP and NCP in shaded streams despite irradiances that clearly placed strong constraints on periphyton production and fell well below irradiances considered growth-saturating (photosaturation occurs at $c. 100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; Hill, Fanta & Roberts 2009). Phosphorus enrichment increased GPP almost two times even at the lowest light treatment where irradiances were $< 20\%$ of saturation and characteristic of highly shaded forest streams that have been considered limited only by light (e.g. Hill, Ryon & Schilling 1995). CR was colimited by light and phosphorus as well, but the respiratory response to resource enrichment was more modest than that of GPP. As a consequence, the NCP increased dramatically with the combined effects of light and phosphorus (Fig. 5).

Heterotrophy prevailed at the lowest light levels despite non-trivial quantities of algal biomass. An allochthonous source of organic matter is required to fuel respiration in heterotrophic environments, and in our experiments, DOM in the water flowing into the streams from First Creek was likely the principal source. First Creek is a typical undisturbed forest stream with DOM concentrations of $c. 1 \text{ mg L}^{-1}$ (W. R. Hill, unpubl. data). Particulate allochthonous organic matter probably contributed little fuel for stream metabolism as leaf litter was non-existent and fine particulate organic matter was not observed entering the streams. The organisms metabolizing the allochthonous organic matter undoubtedly included bacteria, but algal cells may have been utilizing allochthonous DOM as well. The diatom *A. minutissima*, which was particularly common in the streams with low resources, is reported to be facultatively heterotrophic at low irradiances (Tuchman *et al.* 2006). This diatom and possibly others in the streams may have augmented their energy and carbon budgets with stream DOM, contributing more to CR than they would as obligate phototrophs. Because the experimental streams lacked a true hyporheic zone, CR rates were smaller (and NCP rates consequently larger) than reported for most streams (Mulholland *et al.* 2001).

Although bacteria were likely to have had a significant role in community metabolism at low resource levels, their relative importance in the periphyton waned as light and phosphorus increased. Bacterial biovolume failed to increase as quickly as algal biovolume, resulting in a declining ratio of bacteria : algae as primary productivity and periphyton AFDM increased. Similar responses to resource enrichment have been observed in open-water habitats: bacterial biomass and production are much greater relative to phytoplankton in unproductive oligotrophic waters than they are in productive mesotrophic–eutrophic waters (e.g. Biddanda, Ogdahl & Cotner 2001; Duarte *et al.* 2005). A relatively high proportion of nutrients in organic form favours bacteria in oligotrophic pelagic habitats, whereas inorganic nutrients favour phytoplankton (Cotner & Biddanda 2002). Light enrichment also reduces the relative contributions of bacterial biomass and production in the plankton (Duarte *et al.* 2005). Bacterial growth in ben-

thic and open-water habitats may simply be unable to keep pace with algal growth as light and nutrients increase. The maximum potential growth rates of bacteria are certainly much greater than those of algae, but bacteria in unproductive waters appear to grow much slower than phytoplankton (Cotner & Biddanda 2002). Increased grazing of bacteria by protozoans that find both food and shelter in vertically expanding periphyton communities may also constrain bacterial growth relative to that of algae. Our results provided little support for the light : nutrient hypothesis that bacteria are favoured over algae in high-light environments.

Resource supply influenced both the taxonomic composition and the size structure of algae in the experimental streams. Light augmentation decreased the contributions of small diatoms such as *A. minutissima* to algal biovolume and increased the contributions of larger diatoms such as *Synedra acus* and *Melosira varians*. Increasing the cell size with increasing resource availability is consistent with allometric predictions. Smaller species should be favoured under conditions of resource scarcity because their relatively large surface-to-volume ratios enable greater efficiencies in both nutrient uptake and photon absorption, whereas larger species should be better suited to resource abundance because they have relatively high nutrient-uptake capacities and are less susceptible to photoinhibition (Irwin *et al.* 2006; Key *et al.* 2010). Small phytoplankton consistently dominate oligotrophic waters whereas large diatoms are generally associated with high levels of primary production in nutrient-rich oceanic waters (Duarte *et al.* 2000). In periphyton, larger taxa are known to overgrow and shade smaller, non-motile species as biomass accumulates (Steinman, Mulholland & Hill 1992), and in the case of species like *Melosira varians* that lack attachment mechanisms, a threshold level of biomass may be necessary to provide vertical structure in which they can entangle and develop (Hill, Fanta & Roberts 2009).

Although most individual species did not respond strongly to phosphorus enrichment, *Meridion circulare* was an important exception. This colony-forming diatom was many times more abundant in streams *without* added phosphorus than it was in phosphorus-enriched streams. It is unclear why *Meridion circulare* responded so poorly to phosphorus enrichment, but the diatom was certainly successful in unenriched streams, accounting for as much as 20% of algal biovolume. This proportion probably underestimates its importance because only cell volume (and not the copious surrounding mucilage) was used in algal biovolume calculations. *Meridion circulare*'s macroscopic, mucilaginous colonies often exceeded 1 cm in diameter in phosphorus-poor streams receiving full light. These colonies excluded other taxa from stream substrata where they occurred. Higher irradiances appeared to facilitate the diatom's ability to preempt space, perhaps because the production of mucilage requires high rates of carbon fixation.

Several mechanisms potentially underlie the synergistic effects of light and phosphorus on primary production. Phosphorus enrichment is hypothesized to allow individual cells to increase the photosynthetic efficiency at low irradiances (Hessen, Faerovig & Anderson 2002), similar to the role

nitrogen plays in facilitating photoacclimation (Prezelin & Matlick 1983). There is currently limited empirical support for a connection between phosphorus and photoacclimation, but Hessen, Faerovig & Anderson (2002) did report correlations between cell-specific Chl *a* concentrations in phytoplankton and ambient phosphorus concentrations, and they suggested that the synthesis of light-harvesting machinery depends on the availability of phosphorus for lipid manufacture. In our experiments, phosphorus enrichment increased the Chl *a* : AFDM ratio in the periphyton and was likely to have increased photosynthetic efficiency at the medium- and high-light treatments, but enrichment did not raise Chl *a* : AFDM ratio at the lowest light level and could not account for higher rates of primary production in low-light, phosphorus-enriched streams. If phosphorus facilitated primary production at low irradiances through physiological changes in individual cells, it occurred through means other than Chl *a* synthesis.

Heterogeneity in resource availability and utilization can potentially account for resource synergies. Light and nutrients colimit grassland production because these resources occur at the top and the bottom of the community, respectively (Hautier, Niklaus & Hector 2009). In periphyton, resource gradients occur at microscales, but vertical gradients can be very steep over short distances (Dodds, Biggs & Lowe 1999). Algal cells in one part of the periphyton matrix could potentially experience limiting supplies of light but saturating quantities of nutrients whereas cells in another location experience saturating supplies of light and limiting supplies of nutrients. Additions of nutrients and light would stimulate the algal growth in both locations, raising community production beyond what would occur by augmenting just one resource. This explanation is problematic in that light and nutrient gradients within periphyton are often congruent, decreasing synoptically from the water–periphyton interface to the interior of the periphyton community. Nutrient regeneration in deeper portions of the periphyton matrix could theoretically create a nutrient gradient that was the reverse of a light gradient, however, creating conditions more favourable to light–nutrient colimitation.

Changes in algal assemblage composition brought about by light and phosphorus enrichment may offer the best explanation for the synergistic effects of the two resources on stream metabolism. The large algal species that were abundant at higher irradiances are likely to be more effective than smaller species in utilizing higher concentrations of phosphorus because of allometric considerations. As discussed above, large diatoms and high levels of productivity are associated with nutrient enrichment in coastal pelagic zones (Duarte *et al.* 2000). The large diatom *Melosira varians* that was substantially more abundant in our high-light treatments has been specifically linked to nutrient enrichment in streams (Lowe 1974). Selection against the colonial diatom *Meridion circulare* by phosphorus enrichment may have also led to more efficient conversion of light into primary production in phosphorus-enriched streams. Stream algae that form mucilaginous colonies are reported to fix carbon at lower biomass-specific rates than non-mucilaginous taxa, especially

at low irradiances (Steinman, Mulholland & Hill 1992). In any case, the selection of assemblages by the augmentation of one resource appears to have had the consequence of increasing the capacity to exploit the other resource. The ecological selection of species assemblages that are best suited to particular resource combinations is predicted by Danger *et al.* (2009) to be a major force driving communities away from single-resource limitation and towards colimitation. Our interpretation of the relationship between resource-driven changes in algal assemblage structure and stream metabolism is consistent with this prediction. Nonetheless, inferences about causal relationships between assemblage structure and productivity are hypothetical without independent control of species composition.

Relatively few studies have reported the simultaneous limitation of primary production by light and nutrients in aquatic ecosystems. The physiological consequences of simultaneously scarce supplies of light and nutrients have been studied in chemostat experiments and other laboratory studies on unialgal cultures (e.g. Goldman 1986), but ecological discussions of resource limitation have generally focused on single resources (but see Rosemond 1993). Because supplies of light and nutrients often covary (both within and between ecosystems), habitats where both resources are in short supply may be relatively common. Co-occurring deficiencies of light and nutrients are especially the characteristic of headwater streams in undisturbed forests, where shading by streamside trees can be extensive and concentrations of nitrogen and phosphorus concentrations can be quite low (Hill, Mulholland & Marzolf 2001; Binkley *et al.* 2004). Light has often been characterized in highly shaded streams as a resource that is so scarce that it precludes nutrient limitation (Lowe, Golladay & Webster 1986; Hill & Knight 1988; Hill, Ryon & Schilling 1995). Our results indicate that this view is overly simplistic, as nutrient enrichment in even the most shaded of our experimental streams consistently altered both algal assemblage structure and stream metabolism. We suggest a revised view of resource limitation in which the supply of a single resource may constrain primary production, but the combined effects of multiple scarce resources on autotrophic communities are potentially much larger than the effect of any individual resource.

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