

**NEWS AND VIEWS****Perspective**

Testing the light:nutrient hypothesis: Insights into biofilm structure and function using metatranscriptomics

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Abstract

Aquatic biofilms are hotspots of biogeochemical activity due to concentrated microbial biomass (Battin, Kaplan, Newbold, & Hansen, 2003). However, biofilms are often considered a single entity when their role in biogeochemical transformations is assessed, even though these biofilms harbour functionally diverse microbial communities (Battin, Besemer, Bengtsson, Romani, & Packmann, 2016; Veach, Stegen, Brown, Dodds, & Jumpponen, 2016). Often overlooked are the biotic interactions among biofilm components that can affect ecosystem-scale processes such as primary production and nutrient cycling. These interactions are likely to be especially important under resource limitation. Light is a primary resource mediating algal photosynthesis and both phototrophic and heterotrophic production due to bacterial reliance on C-rich algal exudates (Cole, 1982). However, current understanding of function–structure linkages in streams has yet to unravel the relative degree of these microbial feedbacks under resource availability gradients. In this issue of *Molecular Ecology*, Bengtsson, Wagner, Schwab, Urich, and Battin (2018) studied stream biofilm responses to light availability to understand its impact across three domains of life. By integrating biogeochemical rate estimation and metatranscriptomics within a microcosm experiment, they were able to link primary production and nutrient uptake rates to algal and bacterial metabolic processes and specify what taxa contributed to gene expression. Under low light, diatoms and cyanobacteria upregulated photosynthetic machinery and diatom-specific chloroplast rRNA suggesting heightened transcriptional activity under light limitation to maintain phototrophic energy demands. Under high light, heterotrophic bacteria upregulated mRNAs related to phosphorous (P) metabolism while biofilm P uptake increased indicating high bacterial-specific P demand when algal biomass was high. Together, these results indicate that biogeochemical function is mediated by complex microbial interactions across trophic levels.

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Stream biofilms are comprised of diverse microbial consortia including autotrophs, heterotrophic bacteria and eukaryotic consumers (e.g., ciliates, chironomids), yet biofilms are often considered a single entity contributing to large-scale process rates. Studies have

examined interactions among biofilm microbial taxa and determined their effect on ecosystem-scale processes, such as nutrient cycling, but most focus on a coarse functional group representation (e.g., autotrophs vs. heterotrophs) or only focus on dominant microbial taxa or species. The interactions among microbial taxa also change under varying resource availability (e.g., low light and nutrients), and can be examined within the context of the light:nutrient hypothesis. The light:nutrient hypothesis states that when light availability is high relative to nutrients, periphyton become C-rich and nutrient-poor, whereas under low light, periphyton are C-poor and nutrient-rich (Sterner, Elser, Fee, Guildford, & Chrzanowski, 1997). These dynamics indicate that the relative availability of light and nutrients determines algal biofilm nutrient content and resource quality in streams (Fanta, Hill, Smith, & Roberts, 2010; Hill, Fanta, & Roberts, 2009; Hill, Roberts, Francoeur, & Fanta, 2011). High light availability results in C-rich algae exuding dissolved organic matter, which is then taken up by bacteria and fuels their production (Sterner et al., 1997). However, knowledge pertaining to the linkages between microbial gene expression profiles and ecosystem-level processes in combination with specific microbial taxa mediating function is relatively lacking in this context. In this issue of *Molecular Ecology*, Bengtsson et al. (2018) use a suite of biogeochemical and molecular approaches to unravel microbial interactions among stream biofilm components and their responses to varying light conditions. These data provide a mechanistic understanding of the structural contribution to photosynthetic and P-metabolism regulation using a novel

framework that can be readily incorporated into future biogeochemical research.

In Bengtsson et al. (2018), stream biofilms were established in diverted flumes and transferred to laboratory microcosms under three light availability treatments (Figure 1). On average, biofilms were dominated by phototrophs, including cyanobacteria, diatoms and, to a lesser degree, green algae (Figure 2). The authors demonstrated that under light limitation, diatoms and cyanobacteria upregulated photosynthetic machinery by 0.5-fold and diatom-specific chloroplast rRNA by twofold although algal biomass and gross primary production rates were lower compared to biofilms under high-light conditions. Regulation of these putative mRNAs, specifically for electron transport and phosphorylation in diatoms and light-harvesting complexes in cyanobacteria, did not translate into compositional changes of dominant taxa. However, light availability correlated with transitions in “subdominant” microbial community composition (top 50–100 most abundant taxa in small subunit (SSU) rRNA sequences). Interestingly, diatoms dominated algal communities, comprising 30%–35% of SSU rRNA abundance (cyanobacteria were secondarily dominant) across light conditions. There was little change in the relative abundance of diatoms across light availability treatments, suggesting that photosynthetic plasticity occurs for these groups under variable light. The ability to maintain dominance yet regulate this photosynthetic machinery exemplifies algal resiliency to environmental conditions and implicates their importance for biofilm stability.

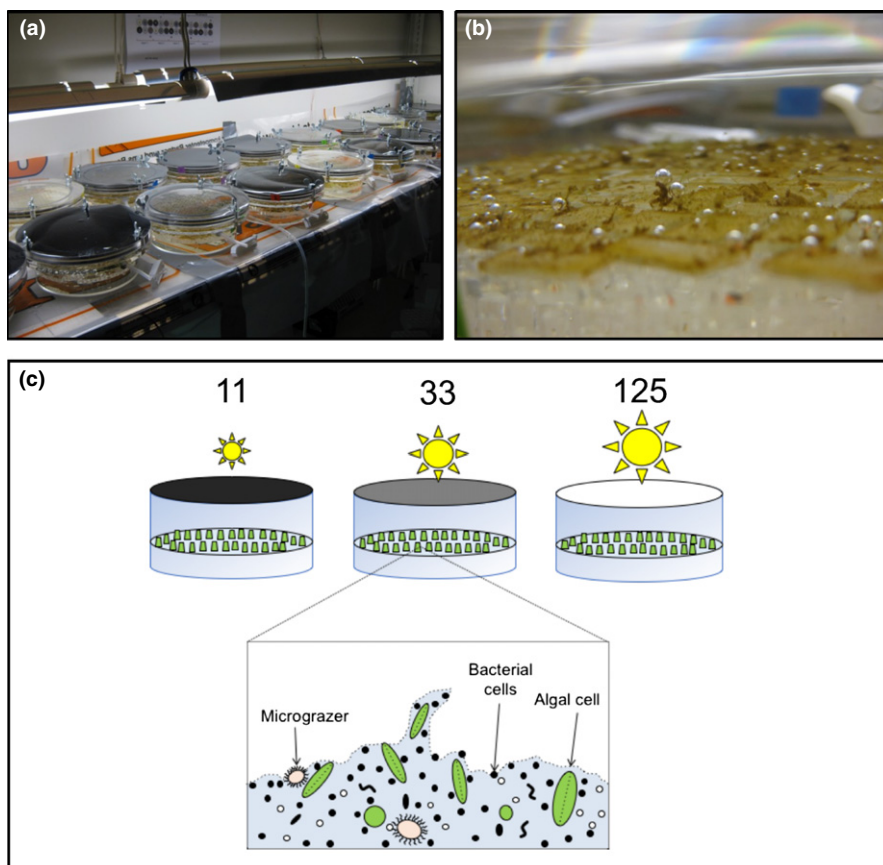


FIGURE 1 Experimental set-up of the Bengtsson et al. (2018) study including plexiglass microcosm chambers equipped with shading foil (a); stream biofilms grown in diverted flumes on glass tiles within microcosms (b); conceptual depiction of three light conditions manipulated above the diverse biofilms: 11, 33 and 125 micromole photons m^2/s^2 with light transmission of 7%, 51% and 92%, respectively (c). Image credits to Mia Bengtsson

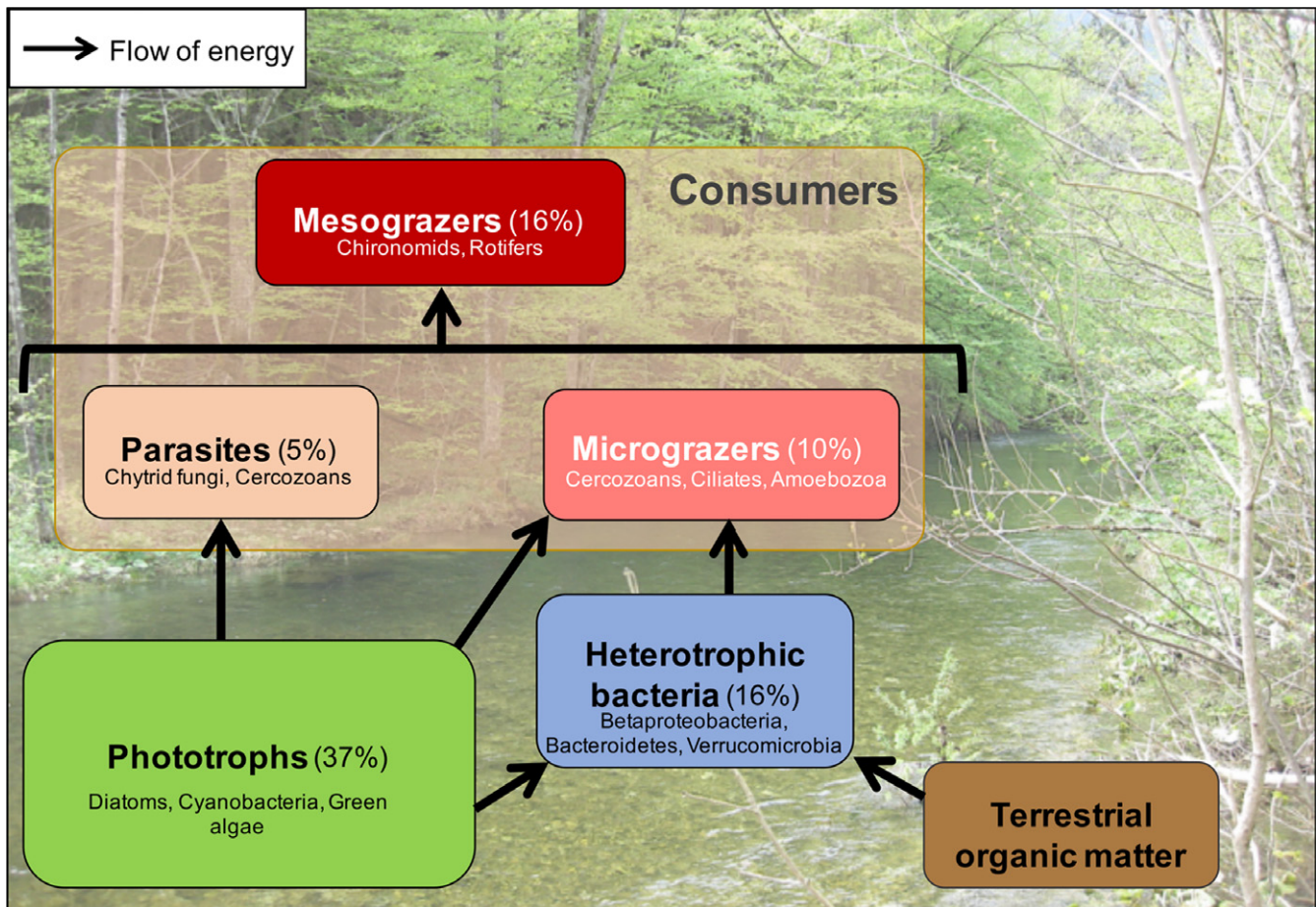


FIGURE 2 Hypothesized microbial food web in Bengtsson et al. (2018) and the proportion of SSU rRNA sequences attributed to each compartment, highlighting the diverse interactions which occur in a stream biofilm. The photograph in the background is the Oberer Seebach stream in Austria, from which the biofilms used in this study were collected. Image and figure credit to Mia Bengtsson

Bengtsson et al. (2018) also found that under high light, P-metabolism mRNA transcripts are upregulated by 1.5-fold and protein functions were expectedly diverse (i.e., transport system regulation, alkaline phosphatase, periplasmic phosphate-binding proteins). These results were mirrored at the “ecosystem” scale as an increase in P uptake, greater microbial biomass and higher primary production rates. These mRNAs were primarily classified as bacterial (both heterotrophic and cyanobacterial), indicative of nutrient depletion relative to bacterial-specific demand. The increased expression of P-metabolism mRNAs was attributed to common bacterial biofilm phyla—*Cyanobacteria*, *Betaproteobacteria* and *Alphaproteobacteria*—yet was independent of their abundance across light availability as these phyla either declined in abundance or remained unchanged with increased light availability. P-metabolism regulation in these biofilms was primarily a physiological response and not necessarily attributed to functional differentiation among bacterial taxa. Together, these results are profound as they evidence a decoupling of function–structure for dominant microbial groups, both algal and bacterial, within biofilms.

Stream biofilm studies commonly target one taxonomic or functional group of microorganisms under limiting resource availability,

yet microbial biofilms constitute several trophic levels. Bengtsson et al. (2018) also found that on average, 15–25% of SSU rRNA sequences were diverse eukaryotic grazers including chironomids (15%), rhizarians (*Cercozoa*—5%), ciliates (2%) and chytrid fungi (2%; Figure 2). At this broad taxonomic scale, micrograzer abundances were stable throughout the light availability gradient contrary to subdominant microbial communities, but specific genera, particularly ciliates, exhibited turnover across light conditions. This effect is likely due to feeding trait selection via differential bacterial prey abundances under low light as heterotrophic bacteria were 0.35-fold more abundant under low-light vs. high-light conditions. The prevalence of eukaryotic consumers within biofilms suggests potential importance of top-down control in stabilizing autotrophic and heterotrophic biofilm communities.

In summary, Bengtsson et al. (2018) contribute to the growing knowledge of structure–function linkages in stream biofilms in key ways. Physiological adaptations in algae, most notably diatoms, and bacteria primarily drove gene expression regulation related to photosynthesis and P-metabolism, respectively. Although community composition overall did not change across light availability, “subdominant” or rather, less abundant taxa (particularly bacteria and certain groups

of micro-consumers) were responsive to changes in resource availability. These findings suggest that in streams where light regimes are spatially heterogeneous, biogeochemical rates will vary due to differential energy flow and microbial stocks, but biofilms are functionally and structurally resilient and capable of essential functions even under limiting resources. The work presented by Bengtsson et al. (2018) is significant as it demonstrates that diatoms and cyanobacteria serve as biofilm ecosystem engineers which facilitate recruitment of hyperdiverse microbiota and are likely maintained long-term through intra-biofilm interactions across multiple trophic levels.

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AUTHOR CONTRIBUTIONS

A.M.V. and N.A.G. wrote the manuscript.

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