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DISSERTATION

Titel der Dissertation

"Are stream biofilms a prime site for priming?"

verfasst von

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1. Summary

Stream ecosystems are sites of intense biogeochemical processing leading to high CO_2 outgassing from inland waters to the atmosphere. Climate change is predicted to lead to increased transport of terrestrial carbon to inland waters, enhancing the emission of greenhouse gases from streams to the atmosphere and thus promoting the global warming trend. Further research is needed to ascertain the fate of this terrestrial carbon in stream ecosystems. Terrestrially derived organic carbon is generally assumed to be of rather low bioavailability. However, research on soil ecosystems found that the availability of labile carbon sources increased the mineralization of the refractory carbon pool, a phenomenon known as the priming effect. In stream biofilms, the dominant form of microbial life in stream ecosystems, phototrophic and heterotrophic microorganisms co-exist in close spatial proximity. The release of photosynthetically derived labile carbon may trigger enhanced degradation of terrestrial refractory organic carbon through priming and thereby contribute to the CO_2 emission from streams. My PhD thesis focused on the investigation of priming effects in benthic and hyporheic freshwater biofilms, as potentially important mechanisms controlling the processing of terrestrially derived carbon in stream ecosystems. I found that the uptake dynamics of refractory terrestrial and labile dissolved organic matter (DOM) by stream biofilm communities did not support priming as a relevant mechanism in stream ecosystems. Generally, co-metabolism strategies, where the energy provided by labile DOM sources is used for extracellular enzyme production and refractory DOM degradation, were apparently of minor importance in these biofilm communities. The lack of priming could be explained by the preferential use of labile DOM over refractory DOM. I found effects, however, of labile and refractory DOM additions on

community composition and function, but not on the diversity for both benthic and hyporheic biofilm communities.

In hyporheic biofilms, refractory DOM lead to a shift in community composition, while labile DOM sources just affected it transiently. Both DOM sources did not affect community function, indicating functional redundancy. However, temporal shifts in the structure of the active hyporheic microbial community was paralleled by a shift in community function. In benthic biofilms, light availability influenced primary productivity and phototrophic DOM release, which altered the composition of the DOM available for heterotrophic growth. This affected the microbial community composition and had also a strong effect on community function. Specific extracellular enzyme activities were found to be correlated with nutrient cycling and community respiration, supporting the link between biofilm structure–function and biogeochemical fluxes in streams. Overall, alpha-diversity did not increase or decrease with the addition of labile organic matter in any stream biofilm community, though hyporheic biofilms showed higher diversity than benthic biofilm communities. This suggests that the resource gradients in hyporheic biofilms fostered higher niche differentiation and decreased interspecific competition compared to benthic biofilms. Summarizing, our observations on the absence of priming in stream biofilms are consistent with other studies that were not able to unequivocally demonstrate priming as a major process in freshwater microbial communities.

2. Introduction

2.1 Effects of climate change on stream ecosystems and carbon cycling

Inland waters are an important integrator of the landscape linking terrestrial ecosystems, groundwater, the atmosphere and the oceans (Battin et al. 2008, Cole et al. 2007). Inland waters are especially threatened by climate change, which is projected to significantly reduce renewable surface water and groundwater resources (Jimenez-Cisneros et al. 2014) and may also affect multiple facets of freshwater ecosystem biodiversity (Heino 2009) and ecosystem functioning (Allison and Martiny 2008, Turner et al. 2000). To evaluate the role of inland waters in the global carbon cycle, a better understanding of the balance between transport, burial and mineralization of organic carbon in streams and rivers is required. In recent years the role of streams and rivers in passively transporting organic carbon from terrestrial soils to the oceans has been challenged (Battin et al. 2008). Now, inland waters have been recognized as important sources of greenhouse gas emissions, and CO_2 outgassing from inland waters to the atmosphere has recently been estimated to amount 2.1 Pg C yr^{-1} (Raymond et al. 2013). In stream networks, small first and second order streams show the highest carbon turnover in the hyporheic zone (Fuss and Smock 1996, Naegeli and Uehlinger 1997), the part of the streambed where ground- and streamwater mix (Boulton 1998). High microbial biomass combined with elevated dissolved organic matter (DOM) retention (Brunke 1997) in the hyporheic zone enables intense biogeochemical processing, which subsequently leads to the net-heterotrophy of these headwater streams (Cole et al. 2007, Battin et al. 2008). In contrast, in larger streams and rivers the major part of the system metabolism takes place in the benthic zone (Edwards et al. 1990), the interface between streamwater and stream sediment. In these benthic habitats algae comprise the major part of the biomass producing organic carbon (Vannote et al. 1980) and therefore often render

the benthic microhabitat autotrophic (Minshall 1978). However, measurements of bacterial production in streams show that they are also fueled to a large extent by other sources than phototrophic primary production like terrestrial carbon inputs (Bott and Kaplan 1985, Findlay et al.1998, Cole et al. 2007). Interestingly, these terrestrial carbon inputs have often been considered refractory and resistant to microbial degradation as they have been stored in soils over extended periods of time (Trumbore 1997). This contrasts with studies showing that terrestrial carbon is actively consumed by stream microbes, thereby contributing to the net-heterotrophy of stream ecosystems (Mayorga et al. 2005, Dodds and Cole 2007, McCallister and delGiorgio 2008). In face of climate change increased carbon loading from the terrestrial habitat to streams is predicted, potentially leading to increased water turbidity (i.e. browning) and altered streamwater DOM concentration and composition (Evans et al. 2006, Roulet et al. 2006). It is still unknown how these changes will affect aquatic microbial communities and their uptake dynamics of refractory terrestrial carbon sources.

2.2 Priming effects in aquatic ecosystems

Stream biofilms dominate the heterotrophic metabolism in many aquatic ecosystems and are the major players in the uptake, storage and transformation of dissolved organic matter and nutrients (Battin et al. 2003, Romani et al., 2004). Until now there is little empirical evidence on the degradation dynamics of labile and refractory DOM in stream biofilm communities. The interaction between C pools with different bioavailability is termed the priming effect (PE), where a change in the mineralization rate of refractory DOM follows inputs of labile DOM. PEs are well studied in terrestrial ecosystems, where plant roots release highly labile DOM into the rhizosphere, which primes the degradation of more refractory DOM by soil microorganisms

(Kuzyakov 2010, Fontaine et al. 2007, Blagodatskaya and Kuzyakov 2008). In the rhizosphere, soil microbial populations show 2-10 times higher microbial biomass next to roots than in the bulk soil (Dijkstra et al. 1987, Paul and Clark 1989, Chen et al.2002). Similarly, the spatial arrangement of stream biofilms may promote priming effects as attached microorganisms within biofilms experience increased residence times of terrestrial carbon, which enhances the potential for the metabolism of more complex substrates (Battin et al. 2008). Furthermore, exudation of labile DOM like carbohydrates and amino acids (Myklestad 1995, Espeland et al. 2001) by phototrophs in stream biofilms enhances bacterial numbers and their growth in the spatial proximity of algae. This substrate co-occurrence in biofilms may foster co-metabolism strategies (Guenet et al. 2010), where microbial cells invest energy from labile carbon uptake into extracellular enzyme production for refractory carbon degradation. Refractory DOM degradation may furthermore drive the release of nutrients (i.e. nutrient mining; Guenet et al. 2010, Kuzyakov et al. 2010), which again may favour growth of the microbial biofilm community.
In contrast, the addition of labile DOM has also been reported to result in a reduction of refractory DOM mineralization (e.g. Gontatiki et al. 2013). This is mostly the result of preferential substrate utilization (PSU), where the bacterial community preferably degrades the labile carbon source and just turns to the more refractory source when the labile carbon source is less abundant (Gontatiki et al. 2013). Priming effects are a widely accepted mechanism in soils after more than 80 years of research (Löhnis et al. 1926), whereas it just recently started to attract scientific interest in freshwater ecosystems, where its importance remains inconclusive (e.g. Catalan et al. 2015, Kuehn et al. 2014, Danger et al. 2013, Franke et al. 2013).

2.3 Implications of priming for the structure and the function of stream biofilm communities

We investigated if different sources of organic carbon and nutrients shape stream biofilm structure, diversity and functioning and if shifts in these parameters are the result of the priming of refractory DOM. We hypothesized that biofilm communities may react in several ways to the presence of labile and refractory DOM sources: When labile carbon sources are temporarily available like during algal blooms, microbial biofilm communities may alter their composition as a response and return to their original composition as soon as the labile DOM is depleted (i.e. community resilience; Allison and Martiny 2008, Milferstedt et al. 2013). Microbial communities may also enter a reversible state of reduced metabolic activity or dormancy when labile DOM sources are scarce (Lennon and Jones 2011). Alternatively, the presence of different DOM sources may induce a permanent shift in community composition. Functional redundancy, where remaining biofilm bacteria modify their niches as others disappear, may allow the microbial community to maintain ecosystem functioning like productivity, carbon and nutrient cycling under changing conditions (MacGillivray 1995, Grime et al. 1997). If remaining bacteria can't modify their niches it may lead to changes in functioning and ecosystem production (Mikola 1998, Allison and Martiny 2008). However, the inability to detect functional consequences emerging from biodiversity loss may often be due to the broad ecosystem processes assessed like microbial respiration, which integrates across many individual processes (Schimel and Schaeffer, 2012). Specific metabolic functions like extracellular enzyme activities are potentially more tightly linked to the composition of a given microbial community (Langenheder et al., 2006). These extracellular enzyme activities may therefore constitute a better measure of biofilm community functioning and provide insight into the use of different

organic carbon sources (e.g. Romani and Sabater 2000, Romani 2004). Unraveling the contribution of different microbial groups and the metabolic pathways they use in DOM degradation may thus broaden our understanding of the priming phenomenon.

3. Aims of the Thesis

3.1 Main goal and experimental approach of the PhD thesis

The main goal of my PhD thesis was to investigate the priming of refractory terrestrial carbon by labile carbon compounds in stream biofilms and its potential implications for C cycling in stream ecosystems. To quantify the priming effect, I used a ^{13}C-labeled willow extract as refractory DOM source, which allowed the calculation of a detailed carbon mass budget and the simultaneous assessment whether any additional respiration after the primer addition could be attributed to enhanced degradation of the refractory DOM source or to degradation of the added primer alone. This experimental approach is relatively novel as most priming studies have used a ^{13}C-labeled primer, which makes the quantification of priming effects more difficult (e.g. Hamer and Marschner 2005, Franke et al. 2013, Van Nugteren et al. 2009). As previously proposed priming may just occur under circumstances where labile primers with a specific composition and concentration are used (Smith et al. 2007). Specifically, more complex labile substrates, such as algal exudates, may be more likely to induce priming as they promote the growth of a wide variety of microbial functional groups (Farjalla et al. 2009, Guenet et al, 2012). We tested this assumption in two environmental contexts: In a first approach we used heterotrophic hyporheic microbial communities and amended them with the refractory DOM source and different labile primers (glucose, glucose+nutrients and an algal extract) treating carbon and nutrient additions as separate sources to identify mechanisms underlying priming (Paper I). In a second approach we investigated if *in situ* algal exudation in benthic biofilms might prime the degradation of the refractory DOM (Paper II&III). Furthermore, we assessed the potential effects of both labile and refractory DOM on community structure, function and diversity (Paper I&II). This is the first

systematic approach to investigate priming effects in stream biofilms and to evaluate their contribution to carbon fluxes in freshwater ecosystems.

3.2 Specific goals of Paper I

Microbial communities in the hyporheic zone receive refractory DOM from terrestrial origin (i.e. allochthonous DOM) and highly labile DOM from in stream production (i.e. autochthonous DOM) via down-welling of algal exudates from the benthic zone. In Paper I, I investigated if these different pools of DOM would drive shifts in the composition, diversity and function of the hyporheic bacterial biofilm community. Specifically, I addressed links between the bulk (based on the 16S rRNA gene) and the metabolically active microbial biofilm community (based on the 16S rRNA), respectively and their links to biofilm function. A separate paper which I co-authored (Bengtsson et al. 2014) quantitatively addressed priming effects from the same experiment.

3.3 Specific goals of Paper II

Clearly, light is a major control on primary productivity in benthic biofilms, regulating phototrophic extracellular release and thereby the quantity of available autochthonous DOM for heterotrophs. In Paper II, I investigated how changes in light availability, and hence in the availability of autochthonous DOM, impact the structure, diversity and functioning of benthic biofilm communities. Furthermore, I aimed to better understand how resource availability affects algal-bacterial interactions and carbon and nutrient cycling within stream biofilms.

3.4 Specific goals of Paper III

The close spatial proximity of microbial heterotrophs and algal cells in stream biofilms may foster the priming of refractory terrestrial DOM by labile DOM stemming from algal exudation and cell lysis. In Paper III, I investigated the resource use of autochthonous and allochthonous DOM by benthic stream biofilm communities using a detailed carbon mass balance and assessing whether the amount of extra CO_2 released originates from increased refractory DOM decomposition or from use of the labile DOM source.

4. Paper I

Functional and structural responses of hyporheic biofilms to varying sources of dissolved organic matter

Wagner K, Bengtsson MM, Besemer K, Sieczko A, Burns NR, Herberg ER, and Battin TJ (2014). Applied and Environmental Microbiology **80**:6004–6012.

My contribution to this study was designing the bioreactor set up, processing samples in the laboratory and generating and analysing the 454-pyrosequencing data in close collaboration with Mia Bengtsson. Additionally, I interpreted the results and wrote the manuscript as first author.

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Functional and Structural Responses of Hyporheic Biofilms to Varying Sources of Dissolved Organic Matter

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Headwater streams are tightly connected with the terrestrial milieu from which they receive deliveries of organic matter, often through the hyporheic zone, the transition between groundwater and streamwater. Dissolved organic matter (DOM) from terrestrial sources (that is, allochthonous) enters the hyporheic zone, where it may mix with DOM from *in situ* production (that is, autochthonous) and where most of the microbial activity takes place. Allochthonous DOM is typically considered resistant to microbial metabolism compared to autochthonous DOM. The composition and functioning of microbial biofilm communities in the hyporheic zone may therefore be controlled by the relative availability of allochthonous and autochthonous DOM, which can have implications for organic matter processing in stream ecosystems. Experimenting with hyporheic biofilms exposed to model allochthonous and autochthonous DOM and using 454 pyrosequencing of the 16S rRNA (targeting the "active" community composition) and of the 16S rRNA gene (targeting the "bulk" community composition), we found that allochthonous DOM may drive shifts in community composition whereas autochthonous DOM seems to affect community composition only transiently. Our results suggest that priority effects based on resource-driven stochasticity shape the community composition in the hyporheic zone. Furthermore, measurements of extracellular enzymatic activities suggest that the additions of allochthonous and autochthonous DOM had no clear effect on the function of the hyporheic biofilms, indicative of functional redundancy. Our findings unravel possible microbial mechanisms that underlie the buffering capacity of the hyporheic zone and that may confer stability to stream ecosystems.

Most headwater streams are net heterotrophic, and allochthonous dissolved organic matter (DOM) is the major subsidy of the heterotrophic metabolism therein (1, 2). It is generally recognized that most of the organic matter processing in streams occurs in the hyporheic zone, which is the part of the streambed where groundwater and streamwater mix (3). The hyporheic zone offers a large surface area for colonization by biofilms, which dominate microbial life in streams and which greatly contribute to DOM retention and transformation (4). High microbial biomass combined with elevated DOM retention renders the hyporheic zone an active compartment of stream ecosystems, even with impacts on large-scale carbon fluxes.

Microbial biofilms in the hyporheic zone encounter allochthonous DOM (AL-DOM) and autochthonous DOM (AU-DOM). For instance, upwelling of shallow groundwater can deliver AL-DOM to streams via the hyporheic zone (3). Furthermore, fresh leaf litter deposited onto the streambed and decaying leaf packs lodged in stream sediments provide a mix of DOM, covering a gradient of bioavailability ranging from labile to semilabile and recalcitrant compounds that can be delivered into the hyporheic zone via downwelling of streamwater. Similarly, AU-DOM from benthic algae can reach the hyporheic zone via downwelling, where it may mix with AL-DOM. In general, such AU-DOM is considered labile to microbial degradation, due to the elevated content of monosaccharides and amino acids (5–7), for instance. On the other hand, AL-DOM, such as leachates from decaying leaf litter, is often aged and depleted in labile compounds and may therefore be resistant (recalcitrant) to microbial degradation (8). The interaction of these different organic matter pools has potentially important implications for carbon cycling in stream ecosystems (9). For instance, labile organic matter may enhance microbial degradation of recalcitrant organic matter through priming or cometabolism (1, 9–11). Therefore, knowledge of the structure and functioning of the microbial communities dwelling in the hyporheic zone and their potential response to variations in the DOM supply is important to better assess the role of the hyporheic zone for biogeochemical processes.

The relationship between environmental controls and community composition and functioning is of central interest for ecology. Both the composition and function of microbial communities have been shown to respond to environmental factors, though it still often remains elusive to what extent changes in community composition are responsible for changes in functioning (12, 13). The manipulation of environmental factors (e.g., DOM quantity and quality) can influence functional and structural community parameters in different ways. Both microbial community structure and function can respond to, or remain unaltered under, environmental influences. Further, the relationship between microbial community composition and functioning may be explained by metabolic plasticity and functional redundancy (14, 15). The former refers to the capacity of a microbial community to adjust its metabolic performance to environmental controls without changing its composition, whereas the latter implies that different taxa can have similar functional roles and the community composition may change while certain functions remain unal-

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tered (14, 15). Furthermore, abundant, dormant, or even dead microorganisms may obscure the relationship between microbial community structure and function. The characterization of microbial community composition by analyses of 16S rRNA bears the potential to circumvent this caveat (16). In fact, RNA has a much shorter life span than DNA and can therefore serve as a tool to capture metabolically active microorganisms.

In this study, we experimentally exposed hyporheic biofilms in bioreactors to AL-DOM extracted from predegraded plant material, simulating allochthonous subsidies to the stream ecosystem. Different amendments simulating AU-DOM sources, including monosaccharides with and without inorganic nutrients (N and P) and algal extracts, were used to mimic the delivery of exudates from benthic algae into the hyporheic zone. A suite of extracellular enzymes was measured as a proxy for microbial function in response to the various DOM sources. We also used 454 pyrosequencing of 16S rRNA and the 16S rRNA gene to study the composition of the bulk and the putatively active microbial communities in the bioreactors. We expected that community composition and extracellular enzyme activities would vary under differing organic substrate conditions. We hypothesized that AU-DOM subsidizing background AL-DOM would affect community composition and function.

MATERIALS AND METHODS

Bioreactor setup and sampling design. Plug-flow glass bioreactors (17) were used to mimic the sedimentary environment of the hyporheic zone. Sintered glass beads (SIRAN carriers, 2- to 3-mm diameter; Jaeger Biotech Engineering) were used as a substratum for biofilm growth from Oberer Seebach (OSB; Lunz am See, Austria, 600 m above sea level). OSB is a prealpine 3rd-order stream draining a pristine, calcareous catchment (approximately 20 km^2) where vegetation is dominated by *Fagus sylvatica* and *Picea abies* and, on the gravel bars of OSB, by *Salix fragilis*. Prior to being packed into the bioreactors, beads were colonized in OSB for 31 days in the dark. This time period was long enough to yield mature microbial communities and typically corresponds to the interstorm interval in OSB. Prior to being packed at equal amounts into bioreactors ($n = 25$), beads were gently rinsed in filtered streamwater (0.2-μm filter) to remove larger debris and invertebrates. Bioreactors were kept in the dark to avoid growth of phototrophs. Bioreactors were fed with filtered OSB streamwater (0.2-μm filter) and amended with DOM (see below) from 4-liter polypropylene copolymer bottles (Nalgene) and operated in a once-through flow mode using peristaltic pumps (Ecoline VC MS/CA2; Ismatec). Each bioreactor was connected to an individual feed bottle. Bottles were replaced roughly every 48 h and autoclaved between changes. The empty bed contact time in the bioreactors averaged 183 ± 11 min, and the flow rate averaged 0.90 ± 0.05 ml min^{-1}. Experiments were carried out at an average temperature of 18.8 ± 1.3°C.

Experimental treatments and phases. We designed four experimental phases, lasting a total of 41 days, to mimic pulsed AU-DOM subsidies from algal exudation on top of a continuous AL-DOM input to hyporheic biofilms. In a first phase, all bioreactors received raw streamwater (1.09 mg C $liter^{-1}$) for 18 days to allow the microbial communities to acclimate to the laboratory conditions. After this acclimation phase, five replicate bioreactors (here termed "starter community") were destructively sampled, and biofilm was collected to determine extracellular enzymatic activities and microbial biomass (including C and N content) and to extract RNA and DNA for 454 pyrosequencing. In a second phase (lasting 2 days), all remaining 20 bioreactors received sterile filtered streamwater (0.2-μm-filter), containing AL-DOM (0.88 mg C $liter^{-1}$) produced from willow (see below). In a third phase (the AU-DOM phase, lasting 7 days), we added model AU-DOM in replicates of five bioreactors as follows: glucose (GLC) (0.44 mg C $liter^{-1}$), glucose and inorganic nutrients (GLC+N+P) (0.44 mg C $liter^{-1}$, 2,187 μg $liter^{-1}$ $N\text{-}NO_3$, and 6.82 μg $liter^{-1}$ $P\text{-}PO_4$), and algal extract (AE) (0.44 mg C $liter^{-1}$, 2,187 μg $liter^{-1}$ $N\text{-}NO_3$, and 6.82 μg $liter^{-1}$ $P\text{-}PO_4$). Five bioreactors receiving streamwater with model AL-DOM, but no AU-DOM, served as control. During this phase, inflow and outflow samples were collected six times for the determination of NO_3, PO_4, O_2, and dissolved organic carbon (DOC) concentrations and three times for the determination of extracellular enzymatic activities. After this AU-DOM addition, we reverted the bioreactors in a fourth phase (the AL-DOM phase, lasting 14 days) to streamwater with AL-DOM (0.88 mg C $liter^{-1}$). After this final phase, biofilms were sampled from all bioreactors for the analysis of extracellular enzymatic activities and microbial biomass and for 454 pyrosequencing.

Inflow and outflow sampling and solute dynamics. Streamwater samples for NO_3, PO_4, DOC, and extracellular enzymatic activity analyses were collected from the inflow and outflow of each bioreactor using three-way valves and syringes (100 ml). Outflow samples were collected at the normal flow rate, while inflow samples were collected at a higher rate by diverting the flow for a short time (<5 min). Samples for NO_3 and PO_4 were analyzed on a continuous flow analyzer (FlowSys 3rd generation; SYSTEA Analytical Technologies). Samples for DOC analysis were filtered (Whatman GFF) and DOC concentrations measured on a TOC Analyzer (Sievers 5310C; GE Analytical Instruments). All glassware was acid washed and combusted. The concentration of dissolved oxygen (DO) at the inflow and the outflow was measured using planar optodes in flow-through cells (PSt3 sensor; Presens, Germany). These measurements were performed 14 times during the experiment.

Oxygen and DOC dynamics. The accumulated mass (M) of O_2 and DOC removed during the AU-DOM and AL-DOM phases of the experiment was calculated according to the following formula:

$$M = \sum_{p1}^{pn} \left(\Delta C_p \times \int_{t_0}^{t_{\text{int}}} Q dt \right)$$

where ΔC_p is the difference in concentration (DOC or O_2) between inflow and outflow of the bioreactor measured at one sampling point, p_1 and p_n refer to the first and last sampling points of a given phase, Q is the flow rate, and t_0 and t_{int} represent the duration of an interval encompassing one sampling point. The mass of O_2 and DOC was normalized to the weight of beads contained in each bioreactor.

Production of model AL-DOM. We produced AL-DOM from a hot-water extraction of crack willow (*Salix fragilis*), which is a common representative of the riparian vegetation along prealpine streams. Leaf and stem material from *S. fragilis* was harvested, dried, and ground (Retch MM2) to a fine powder, which was then extracted in MilliQ water at 95°C (60 min). To remove the labile moieties from the willow extract, we subjected the extract to degradation in 15-liter bioreactors with biofilms growing on porous ceramic carriers (Eheim Mech). Bioreactors were continuously aerated to ensure oxic conditions. The decrease in DOC concentration was monitored over 12 days, and we terminated the degradation at day 12 because no significant decrease in DOC concentration was observed during two consecutive days (see Fig. S1 in the supplemental material). At that point, the willow extract was considered relatively resistant to further degradation, and we used this fraction as AL-DOM (filtered on 0.2-μm filters and stored at 4°C). A similar approach was used in previous work (18).

Microbial biomass and cell abundance. Samples for bulk microbial biomass were harvested from the glass beads by sonication and vortexing, and C and N content was determined using an elemental analyzer (EA1110; CE Instruments, ThermoFisher) (19). An extra sample of glass beads was collected into sterile vials containing 5 ml formaldehyde (2.5%) pending further processing for cell counts. Within 3 weeks after sampling, 20 ml pyrophosphate (0.025 mM pyrophosphate, 2.5% formaldehyde) was added to the glass beads, which were then shaken (60 min) and sonicated three times for 20 s (14% amplitude) to detach the cells from the glass beads. Larger particles were allowed to settle from the supernatant for 20 min. We stained the nucleic acids using SYTOX Green (Life Tech-

nologies Corporation) (5 μM final concentration, 15 min), and microbial cells were counted (Quanta; Beckman Coulter) in aliquots.

Extracellular enzymatic activity. The potential activity of nine extracellular enzymes was measured on the biofilms harvested at the start and at the end of the experiment, as well as in the water from the bioreactor outflow, using substrates linked to aminomethylcoumarin (AMC) and methylumbelliferyl (MUF), respectively, as fluorophores or 3,4-dihydroxyphenylalanine (L-DOPA). All substrates and buffers were purchased from Sigma-Aldrich Chemical Company. The extracellular enzymes were chosen according to their relevance in C, N, and P acquisition (20–23): β-D-glucosidase (EC 3.2.1.21), α-D-glucosidase (EC 3.2.1.20), β-D-xylosidase (EC 3.2.1.37), and cellobiohydrolase (EC 3.2.1.91) are involved in carbohydrate metabolism; leucine-aminopeptidase (EC 3.4.11.1) and endopeptidase (EC 3.4.21-24) are involved in peptide decomposition; phosphatase (EC 3.1.3) is involved in phosphate acquisition; esterase (EC 3.1) breaks ester bonds and serves as a general descriptor of organic matter degradation; and phenol oxidase (EC 1.14.18.1) relates to the hydrolysis of recalcitrant compounds. All fluorogenic substrates were dissolved in 2-ethoxyethanol (Cellosolve), except L-DOPA, which was dissolved in sodium carbonate buffer. The pH of the buffers was set to 7, which approximates the pH of OSB streamwater. MUF and AMC reference standards were prepared with autoclaved MilliQ water. Saturation curves for each enzyme were made to determine the saturation conditions. Glass beads were placed into a sterile Falcon tube (BD Biosciences), and 10 ml of 0.2-μm filtered streamwater from the respective bioreactors was added. Samples were gently sonicated (1 min, 14% amplitude, 1-s pulse; Branson Digital Sonifier 250) to detach and homogenize the microorganisms. We then transferred triplicate aliquots (200 μl) of the biofilm homogenate and the water samples from the bioreactor outflow, respectively, into black 96-well plates (Greiner Bio One) and added 50 μl of the respective substrates. All assays were conducted under substrate saturation conditions and incubated at 19°C in the dark. The fluorescence of the MUF ($EX_{365\ nm}/EM_{455\ nm}$) and of the AMC ($EX_{364\ nm}/EM_{445\ nm}$) was determined on a TECAN Infinite M200 microplate reader. The absorbance for phenol oxidase activity was measured at 450 nm. Fluorescence and absorbance were repeatedly measured over a period of 0.5 to 12 h on a microplate reader (24). For each enzyme, a negative control for the substrate color (0.2-μm filtered streamwater and the respective substrate) was used to account for abiotic degradation of the substrates. Each 96-well plate also included a reference standard using MUF and AMC in various concentrations. Extracellular enzyme activities were expressed as pmol substrate g^{-1} beads h^{-1}. To compare extracellular enzyme activities in the water samples among the AU-DOM and the AL-DOM phase, extracellular enzyme activities were integrated over the phase exposure time (25).

Microbial community analysis. 454 pyrosequencing on the 16S rRNA and the 16S rRNA gene served to assess composition and diversity of the putatively active and bulk community, respectively. Total nucleic acids were extracted from the heterotrophic biofilm following the protocol by Urich et al. (26). Briefly, cetyltrimethylammonium bromide (CTAB) buffer and phenol-chloroform-isoamyl alcohol (25:24:1, pH 6.8) were added to a 15-ml lysing matrix E tube (MP Biomedicals LLC) containing 7 g of glass beads with the attached microbial communities. The cells were lysed in a FastPrep machine (MP Biomedicals LLC), followed by nucleic acid precipitation with polyethylene glycol (PEG) 8000. Genomic DNA was digested using the RQ1 RNase-free DNase kit (Promega Corporation) according to the manufacturer's recommendations. An aliquot of the nucleic acid extract was subjected to DNA digestion with RQ1 RNase-free DNase and incubated in a thermal cycler for 30 min at 37°C. Stop solution was added, followed by incubation in a thermal cycler at 65°C for 10 min. The tube was put on ice, and RNA was purified using the MEGAclear kit (Ambion Life Technologies Corporation) according to the manufacturer's recommendations. Absence of DNA was verified by PCR as described below. RNA was transcribed into cDNA by reverse transcription at 42°C for 60 min using RevertAid reverse transcriptase (Thermo Fisher Scientific Inc.) and random hexamer primers (Thermo Fisher Scientific Inc.), followed by an enzyme inactivation step at 70°C for 10 min. Samples without reverse transcriptase and with MilliQ water instead of target RNA served as negative controls. The hypervariable regions V3 and V4 of the bacterial 16S rRNA gene were PCR amplified using the barcoded forward primer 341F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer 805R (5′-GACTACHVGGGTATCTAATCC-3′) (27). For each sample, two different bar codes were used to reduce bar code-specific bias (28). The DNA and cDNA concentrations of the samples were determined using the fluorescent, DNA binding QuantiFluor double-stranded DNA (dsDNA) system kit (Promega Corporation). Equal template concentrations of DNA and cDNA were amplified in all PCRs. Each 25-μl PCR mixture contained each primer at 0.5 mmol $liter^{-1}$ (Thermo Fisher Scientific Inc.), deoxynucleoside triphosphates (dNTPs) at 0.25 mmol $liter^{-1}$ (Thermo Fisher Scientific Inc.), bovine serum albumin at 40 mg $liter^{-1}$ (Thermo Fisher Scientific Inc.), $MgCl_2$ at 2.5 mmol $liter^{-1}$ (Thermo Fisher Scientific Inc.), and 1 U Taq-DNA polymerase with the recommended PCR buffer (Thermo Fisher Scientific Inc.). Samples were amplified using an initial denaturing step at 94°C for 2 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing for 30 s starting with a 62°C annealing temperature and decreasing to 50°C (reduction of 0.5°C per cycle), elongation at 72°C for 1 min, and a final elongation at 72°C for 10 min. Each PCR included a negative control. PCR products were run on a 1.5% agarose gel (Top Vision Agarose; Thermo Fisher Scientific Inc.). The PCR bands were cut from the gel and purified using the gel extraction kit (Qiagen) according to the manufacturer's recommendations. The purified PCR products were quantified on a 1.5% agarose gel using the Gel Doc XR+System (Bio-Rad Laboratories, Hercules, CA, USA) in combination with the MassRuler DNA Ladder Mix and the recommended loading dye (Thermo Fisher Scientific Inc.). The amplicon concentrations obtained by gel quantification were verified by using the DNA binding QuantiFluor dsDNA system kit (Promega Corporation). Amplicons were pooled in equimolar concentrations and sequenced on a GS FLX titanium sequencer at the Center for Genomic Research (University of Liverpool, Liverpool, United Kingdom).

Statistical analyses. All statistical analyses were performed using the software and statistical computing environment R (R Development Core Team, 2013). Significant differences in removal of oxygen and DOC between the treatments and the control were tested using ANOVA and post hoc Tukey's test. Extracellular enzymatic activities were tested using the nonparametric Kruskal-Wallis test and the Mann-Whitney U test for pairwise comparisons ($P < 0.05$). The significance value was adjusted for multiple comparisons using the Bonferroni correction. A Mantel test and partial Mantel's test with Spearman's rank correlation with 999 permutations were performed to test correlations between environmental variables (NO_3, PO_4, DOC, and O_2 concentrations at the inflow of the bioreactors), species composition, and the respective extracellular enzyme rates.

The 454 pyrosequencing data were denoised, and reads were clustered at a 97% identity level to operational taxonomic units (OTUs) using the software package AmpliconNoise V1.28 (29). Taxonomic assignments were determined using the CREST classifier (30). Alpha diversity was calculated as richness and the number equivalents of the Shannon and of the Gini-Simpson indices, which differently weigh abundant and rare species (31). All samples were rarefied to the lowest number of reads obtained from a sample (2,331 reads) prior to analysis. We computed similarity matrices using the Horn index (32) from randomly resampled 454 pyrosequencing data (33) and from the extracellular enzyme activities, respectively. These similarity matrices were subjected to multidimensional scaling (MDS) analysis to visualize community and functional dynamics of the biofilm bacteria. Permutational multivariate ANOVA (PERMANOVA) was used to test significant differences among the treatments. Alpha diversity measures were tested for significant differences between treatments using ANOVA and post hoc Tukey's test. Differences in beta diversity (variability in community composition between bioreac-

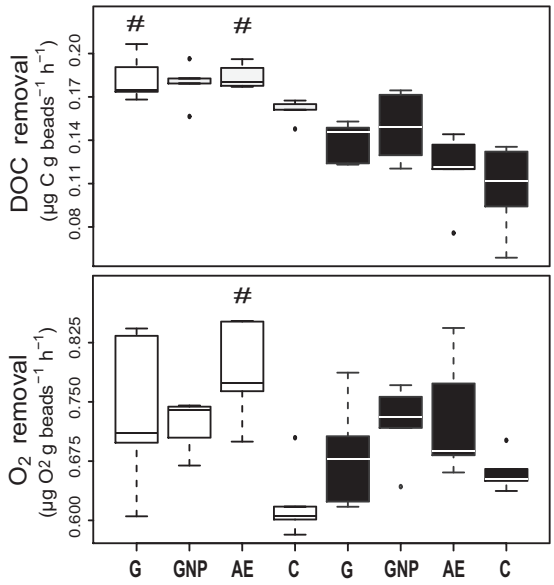

FIG 1 Temporal dynamics of DOC and O_2 removal cumulated over the AU-DOM phase (white) and the AL-DOM phase (black) of the different treatments. Treatments are marked as follows in all graphs: S, starter community; G, GLC; GNP, GLC+N+P; AE, algae; C, control. Outliers are displayed as dots. A hash tag (#) indicates significant differences ($P < 0.05$) between the control and the AU-DOM treatments (G, GNP, and AE).

tors) were assessed by calculating the distance to centroid of each treatment in multidimensional space (34, 35).

Nucleotide sequence accession numbers. The following accession numbers were obtained upon submission of the sequences to the GenBank database: SRX433107 and SRX462313.

RESULTS

Dynamics of dissolved organic carbon and oxygen in the bioreactors. DOC removal was significantly lower in the control, the GLC, and in the algae (AE) treatments during the AL-DOM phase ($P < 0.05$) than during the AU-DOM phase (Fig. 1). DOC removal was significantly lower in the control treatment than in the GLC and the AE treatments during the AU-DOM phase ($P < 0.05$), whereas DOC removal did not differ in the AL-DOM phase among treatments ($P = 0.07$). The removal of oxygen in the bioreactors was significantly lower in the control treatment than in the AE treatment during the AU-DOM phase ($P < 0.01$), while it did not differ among treatments during the AL-DOM phase ($P = 0.24$).

Extracellular enzyme activities in the bioreactor outflow. Phenol oxidase activity was significantly lower ($P < 0.001$) in the control, the GLC, the GLC+N+P, and the AE treatments during the AL-DOM phase than during the AU-DOM phase (Fig. 2). Esterase, phosphatase, and leucine-aminopeptidase activities did not show significant differences between the control, the GLC, the GLC+N+P, and the AE treatments during the AU-DOM and the AL-DOM phase, respectively ($P = 0.99$). Endopeptidase activity was significantly lower ($P < 0.05$) in the control treatment during the AL-DOM phase than during the AU-DOM phase. Endopeptidase activity was significantly higher in the control than in the AE treatment during the AU-DOM phase ($P < 0.05$), whereas endopeptidase activity did not significantly ($P = 0.13$) differ among treatments during the AL-DOM phase.

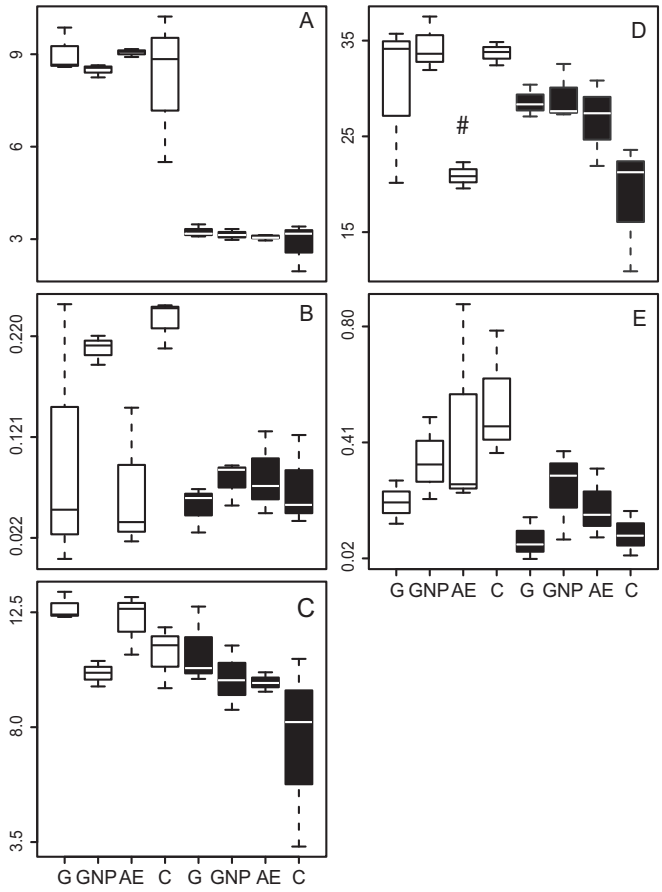

FIG 2 Extracellular enzyme activity (EEAs) of 5 different enzymes cumulated over the AU-DOM phase (white) and the AL-DOM phase (black) of the different treatments (see Fig. 1 legend for abbreviations and significance labeling). Displayed are phenol oxidase activity (A), leucine-aminopeptidase activity (B), esterase activity (C), endopeptidase activity (D), and phosphatase activity (E) as nmol substrate converted h^{-1}.

Microbial biomass. Abundance of microbial cells attached to the beads was significantly lower in the starter community than in the GLC, the GLC+N+P, the AE, and the control treatments ($P < 0.001$) (Table 1). Biomass (total C and N content) of the biofilms did not show significant differences among treatments ($P = 0.81$).

Community composition and diversity. The nonmetric MDS (NMDS) revealed a clear separation between bulk and active community compositions ($P < 0.001$) (Fig. 3). The starter community differed significantly in both bulk and active community compositions from the GLC, the GLC+N+P, the AE, and the control

TABLE 1 Cell abundance and total C and N content of the biofilm biomass[a]

Sample	No. of cells (10^4)	C content (mg)	N content (mg)
Starter community	38.75 ± 5.60	0.072 ± 0.02	0.008 ± 0.002
GLC	79.40 ± 11.30*	0.114 ± 0.03	0.013 ± 0.005
GLC+N+P	71.40 ± 10.21*	0.103 ± 0.02	0.012 ± 0.003
Algae	66.20 ± 11.80*	0.092 ± 0.01	0.011 ± 0.001
Control	70.44 ± 9.00*	0.083 ± 0.02	0.009 ± 0.002

[a] Values are means ± standard deviations per g of beads. *, significant difference ($P < 0.05$) between the starter community and the experimental treatments.

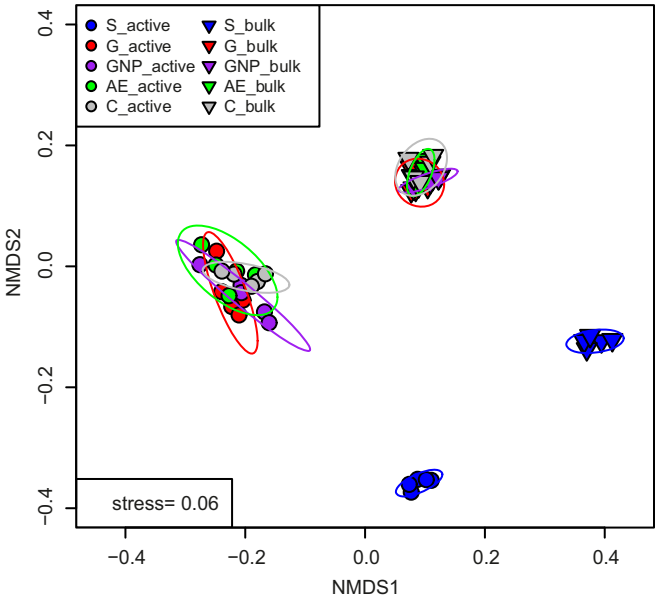

FIG 3 Nonmetric multidimensional scaling (NMDS) ordination based on the Horn distance of the bulk (triangles) and the active community composition (circles) of each bioreactor from the different treatments (see Fig. 1 legend for abbreviations). Symbols are grouped with an ordiellipse (95% confidence interval) to illustrate how treatments cluster in NMDS space.

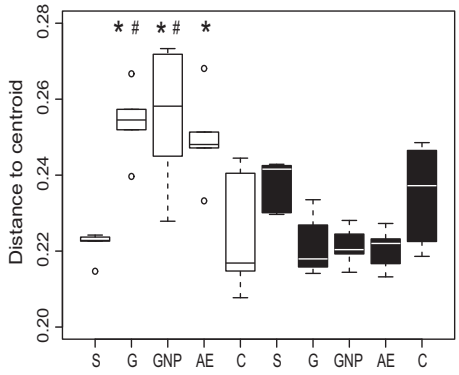

FIG 4 Beta diversity for the active (left panel, white) and the bulk (right panel, black) community compositions under the different treatments. See Fig. 1 legend for abbreviations; *, significant difference ($P < 0.05$) between the starter community and the treatments (G, GNP, AE, and C); #, significant differences ($P < 0.05$) between the control and the AU-DOM treatments (G, GNP, and AE). Outliers are displayed as dots.

treatments ($P < 0.001$). The community composition of the control treatment did not differ from the GLC, the GLC+N+P, and the AE treatments in the bulk ($P = 0.12$) and in the active ($P = 0.77$) communities. Beta diversity (i.e., distance to centroid in NMDS space) of the active starter community was significantly higher than that of the GLC, GLC+N+P, and AE treatments ($P < 0.05$) (Fig. 4). Furthermore, beta diversity of the control treatment was significantly lower in the active community than that of the GLC and the GLC+N+P treatments ($P < 0.05$). The beta diversity of the bulk starter community was significantly higher than that from the AE treatment ($P < 0.05$) (Fig. 4).

Diversity was generally high in the bioreactors. The starter community showed significantly higher richness (as OTUs) than the GLC, the AE, and the control treatments in the active community ($P < 0.05$), whereas the starter community showed significantly higher richness than the GLC, the GLC+N+P, and the AE treatments ($P < 0.05$) in the bulk community. Evenness of the active communities was significantly higher in the starter community than in the AE treatments and the control ($P < 0.05$), whereas no difference in evenness of bulk communities was detected among treatments. Similar trends were observed when comparing the Shannon and Gini-Simpson diversity indices for active and bulk communities between the starter community and the treatments (Table 2).

Overall, we detected sequences from 50 different phyla. The most abundant phylum in the bulk and the active communities was *Proteobacteria* (40.1% ± 2.4% and 44.4% ± 4.8%, respectively). Several of the bacterial phyla displayed shifts in abundance from the starter community to the experimental treatments that were sampled at the end of the experiment. In both the bulk and the active communities, the relative abundance of *Betaproteobacteria* and *Planctomycetes* was significantly ($P < 0.05$) lower in the starter community than in the treatments, whereas *Verrucomicrobia* and *Acidobacteria* increased in abundance. In addition, a significant ($P < 0.05$) decrease in abundance for *Bacteriodetes* and an increase for *Chloroflexi* could be detected in the bulk community but not in the active community. There was no significant difference in abundance of any phylum between the AU-DOM treatments and the control treatment. At the genus level, several taxa displayed shifts in the active and the bulk communities between the starter community and the various treatments (see Fig. S2 in the supplemental material). For instance, *Prosthecobacter* (*Verrucomicrobia*) was the most abundantly assigned genus in our data set and was relatively more abundant in the active than in the bulk community; *Gemmata* and *Hirschia* did not follow that pattern (Fig. 5). Our data show that *Gemmata* exhibited higher relative abundance upon AU-DOM and notably upon algal extract amendments; *Planctomyces* showed an inverse pattern (Fig. 5).

Extracellular enzyme activities of the hyporheic biofilm community. Phosphatase and endopeptidase displayed significantly higher activities in the starter community than in all treatments and in the control ($P < 0.05$) (Fig. 6). However, phosphatase and endopeptidase activities did not differ between the control and the treatments ($P = 0.88$). The activities of the other extracellular enzymes did not show significant differences among treatments ($P = 0.70$).

All treatments displayed similar enzyme activities in NMDS space ($P = 0.56$) (Fig. 7). The starter community overlapped the GLC, the GLC+N+P, the AE, and the control treatments. This indicates a higher overlap on the functional level (Fig. 7) than observed for the community composition (Fig. 3). A Mantel's test showed that environmental variables (NO_3, PO_4, DOC, and O_2 concentrations in the inflow) were significantly correlated with the composition of the active ($R = 0.64$, $P < 0.001$) and the bulk ($R = 0.64$, $P < 0.001$) communities. The active community composition significantly correlated with the extracellular enzyme activities ($R = 0.23$, $P < 0.05$). This pattern was consistent when a partial Mantel's test was performed to test for the correlation between the active community composition and the enzymatic activity, while controlling for the effect of the environmental variables ($R = 0.23$, $P < 0.05$). On the other hand, neither the environmental variables nor the bulk community composition showed any correlation with the extracellular enzyme rates ($R =$

TABLE 2 Characteristics of bulk and active communities under different treatments[a]

Sample	Gini-Simpson NE	Shannon index NE	Richness	Evenness
Bulk community				
Starter community	265.5 ± 22.2	555.0 ± 23.7	994.1 ± 24.3	0.92 ± 0.004
GLC	232.3 ± 28.4	510.3 ± 23.3	920.5 ± 19.6*	0.91 ± 0.005
GLC+N+P	259.4 ± 30.5	528.8 ± 31.7	928.9 ± 30.2*	0.92 ± 0.005
Algae	215.0 ± 36.4	494.3 ± 23.4*	919.0 ± 26.5*	0.91 ± 0.006
Control	234.1 ± 54.4	525.8 ± 44.6	953.0 ± 35.3	0.91 ± 0.008
Active community				
Starter community	207.0 ± 9.6	481.6 ± 11.0	946.3 ± 12.1	0.90 ± 0.002
GLC	79.9 ± 23.9*	356.0 ± 68.8*	869.5 ± 55.0*	0.87 ± 0.026
GLC+N+P	97.2 ± 51.1*	383.2 ± 100.5	875.7 ± 57.2	0.87 ± 0.034
Algae	63.8 ± 15.9*	315.0 ± 38.3*	821.6 ± 35.7*	0.86 ± 0.015*
Control	75.3 ± 18.9*	327.4 ± 44.4*	830.9 ± 18.1*	0.86 ± 0.018*

[a] Values are means ± standard deviations. NE, number equivalents; *, significant difference ($P < 0.05$) between the starter community and the experimental treatments.

0.15, $P = 0.18$). The active ($R = 0.17$, $P = 0.13$) and the bulk ($R = 0.06$, $P = 0.35$) community compositions did not significantly correlate with the extracellular enzyme activities and the environmental variables when the starter community was omitted from the analysis.

DISCUSSION

Hyporheic microorganisms receive AL-DOM, which has often undergone degradation and transformation before entering streams and is therefore considered less labile than AU-DOM (36, 37). The degradation of this AL-DOM may greatly contribute to carbon cycling of streams and may even have potential impact on large-scale carbon fluxes (3). However, little is known on the microbial communities that are involved in these biogeochemical processes and their underlying mechanisms. Our study illuminates the relationship between biofilm structure-function coupling and the processing of AU-DOM and AL-DOM in bioreactors mimicking the hyporheic zone.

Despite the increased metabolism upon AU-DOM additions, there were no corresponding changes in extracellular enzyme activities measured in the outflow of the bioreactors. We expected that AU-DOM provides easily available energy that would enable microorganisms to express more extracellular enzymes enhancing AL-DOM degradation (9). The tendency of higher esterase and endopeptidase activities in the AU-DOM treatments indicates a delayed expression increase of these enzymes. However, as these differences were not significant and no similar effect was observed for these enzymes on the biofilm biomass, priming may not be of relevance in the hyporheic zone. This is consistent with a parallel study on carbon fluxes in the same system (41).

We expected that varying DOM sources differentially affect community composition of hyporheic biofilms. No obvious effect of the autochthonous pulse could be detected on community composition of either the active or the bulk community in response to AU-DOM additions. There may be several explanations for this. Although all DOM additions (including glucose) represented a manipulation within the range of seasonal variation in the OSB streamwater, it might not have been pronounced enough to induce a diversion between treatments and the control. It is likely that concentrations within or directly adjacent to biofilms can be considerably higher than in the streamwater, for example, during a benthic algal bloom. Also, we recognize that we can compare community composition only after the AL-DOM phase and that we cannot draw any conclusions on community dynamics prior to that. It is possible that a shift in community composition occurred directly after the AU-DOM pulse and that the communities converged again to a more similar state until we sampled them at the end of the AL-DOM phase. Another possibility for the relatively minor effect of the AU-DOM pulse on community composition could be that labile DOM is easily metabolized without prior enzymatic degradation by most microbes, thereby not necessitating a change in community composition for an efficient exploitation of these resources.

The importance of AL-DOM for aquatic microorganisms has been shown previously; it is generally believed that AL-DOM provides a relatively continuous energy source that supports slow but steady microbial growth independent of AU-DOM pulses during algal blooms, for instance (37). The consistent divergence in community composition from the starter community that we observed in all treatments is likely attributable to the additions of AL-DOM. We suggest that AL-DOM rather than AU-DOM drives the community dynamics in hyporheic biofilms.

Beta diversity can be related to ecosystem productivity when stochastic community assembly driven by priority effects leads to a higher variability of community composition in productive systems (38). Underlying this notion is the fact that efficient colonizers outcompete others and that various species can thrive in productive ecosystems where community assembly becomes therefore random. We propose that an elevated beta diversity of the active community after the AU-DOM addition is possibly related to such priority effects. As microorganisms encounter presumably labile AU-DOM, stochasticity may drive community assembly, and those taxa that are able to quickly exploit the new resource soon become abundant. Due to priority effects, different microorganisms grow abundant in the different bioreactors, leading to the observed variation in composition. This assumption is supported by elevated beta diversity paralleling increased DOC and oxygen removal as proxies for biofilm metabolism.

We found that the composition between the bulk and the active communities differed markedly, which is likely attributable to various contributions from microorganisms with differing physiological states (39, 42). This may also reflect the varying provenience of microorganisms mixing from various terrestrial and aquatic (including groundwater) habitats in the hyporheic zone. Both the active and bulk communities depicted clear composi-

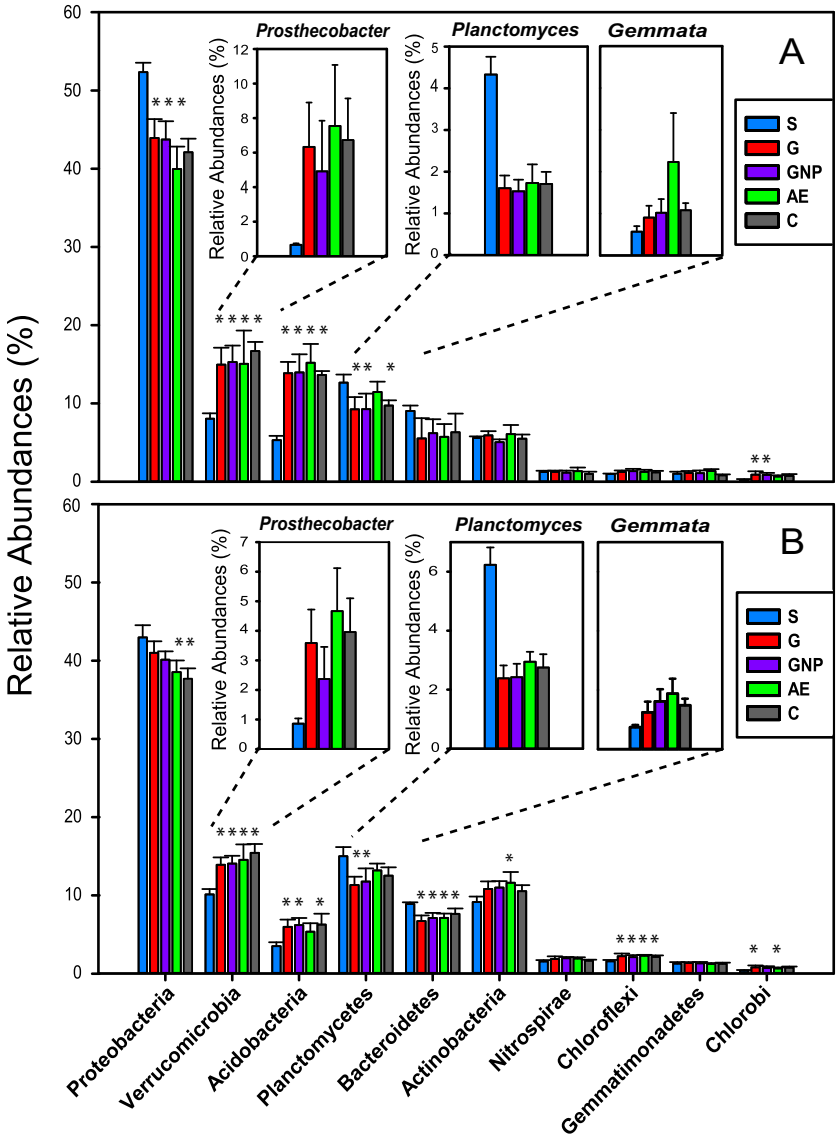

FIG 5 Taxonomic affiliation of the active (A) and the bulk (B) community composition under the different treatments as determined by 454 pyrosequencing. See Fig. 1 legend for abbreviations. Asterisks above the bars indicate a significant difference ($P < 0.05$) between the starter community and the experimental treatments. The most abundant phyla and 3 specific genera are displayed. Unassigned sequences are not shown here and accounted for less than 1% of relative abundance at the phylum level and for approximately 60% at the genus level.

tional shifts at the phylum level between the starter communities and the treatments. This is notable, as it demonstrates an ecological response even at a higher taxonomic rank (43) to relatively small environmental changes. Obviously, the *Proteobacteria* were the most responsive phylum, with the genus *Hirschia* from the *Alphaproteobacteria* providing a good example for a remarkable shift in relative abundance. *Hirschia* was reported to degrade various monosaccharides, amino acids, sugar alcohols, and even cellobiose as suggested by elevated β-glucosidase activity (40). These compounds generally characterize autochthonous and allochthonous DOM, respectively, in aquatic ecosystems and assumedly also in the hyporheic zone, where they mix.

We found different responses of community composition and function (that is, extracellular enzymatic activities) of the hyporheic biofilms to AU-DOM amendments. This is indicative of functional redundancy as has been previously reported from freshwater ecosystems, yet with differing levels of relevance (44, 48); Frossard et al. (45) even reported a clear disconnect between microbial community structure and enzymatic activities. We further investigated the structure-function coupling of hyporheic biofilms by exploring the relationship between bulk or active community and extracellular enzymatic activities. We expected that the environment could influence the microbial community composition with consequences for the extracellular enzymatic activities. The fact that we found a relationship between the active community composition and the respective extracellular enzyme activities, while controlling for the effect of the environment, underlines the link between the active community and enzyme expression. This seems reasonable, given that transient physiological states of microorganisms are considered to be a mechanism that

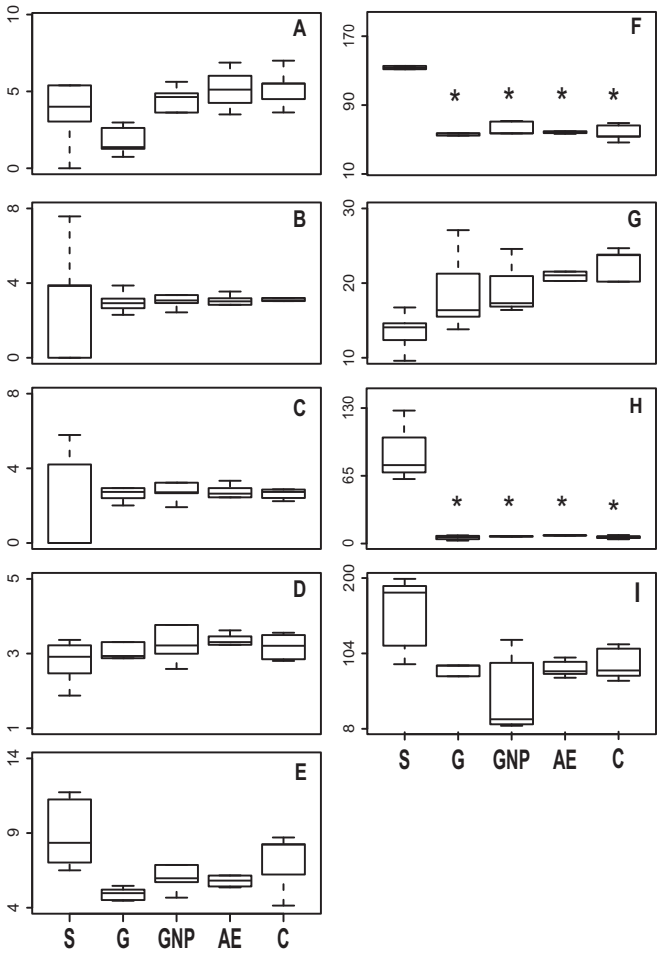

FIG 6 Extracellular enzyme activities of 9 different enzymes from the hyporheic biofilm communities (see Fig. 1 legend for abbreviations; *, $P < 0.05$). Displayed are phenol oxidase activity (A), xylosidase activity (B), cellobiosidase activity (C), α-glucosidase activity (D), β-glucosidase activity (E), endopeptidase activity (F), leucine-aminopeptidase activity (G), phosphatase activity (H), and esterase activity (I) as pmol substrate converted g^{-1} beads h^{-1}. Outliers are displayed as dots.

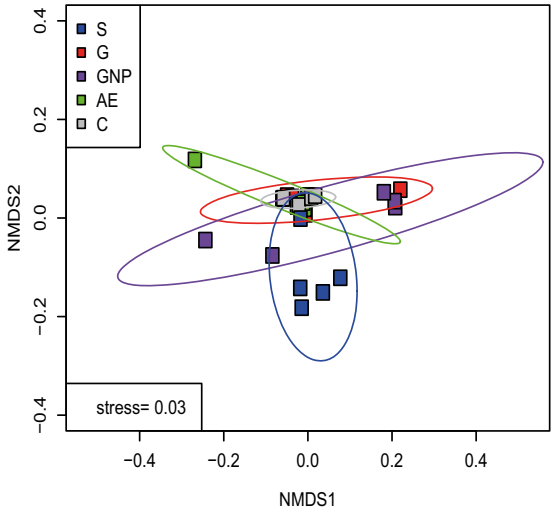

FIG 7 NMDS ordination based on Horn distance of 9 extracellular enzyme activities from each bioreactor of the different treatments (see Fig. 1 legend for abbreviations). Symbols are grouped with an ordiellipse (95% confidence interval) to illustrate how treatments cluster in NMDS space.

contributes to the buffering against environmental fluctuations, including resource availability, and to the maintenance of microbial diversity and functioning (46, 47). Few studies have addressed structure-function coupling of hyporheic microorganisms (12, 19), which our study expands now by showing the apparent need to consider the active rather than the bulk community. No clear relationship between community structure and function existed when the starter community was omitted from the analysis, indicating that the observed relationship is driven by the temporal dimension in our experiment.

It is commonly believed that the hyporheic zone is buffered against environmental fluctuations and that it may contribute to the stability of the ecological and biogeochemical processes of the stream ecosystem (3). Our experimental work unravels microbial mechanisms that potentially contribute to the buffering capacity of the hyporheic zone. For instance, a high taxon turnover (that is, beta diversity) and related priority effects and stochastic community assembly are beneficial in an ecosystem that is characterized by unpredictable hydrology and subsidies of DOM differing in composition and bioavailability. Furthermore, functional redundancy may ensure the continuous provision of essential ecosystem functions independent of community composition, which itself depends on varying environmental processes. Finally, the pool of putatively nonactive microorganisms can sustain the active community as environmental conditions change to favor such microorganisms, which in turn may then upon reactivation sustain critical functions in stream ecosystems.

ACKNOWLEDGMENTS

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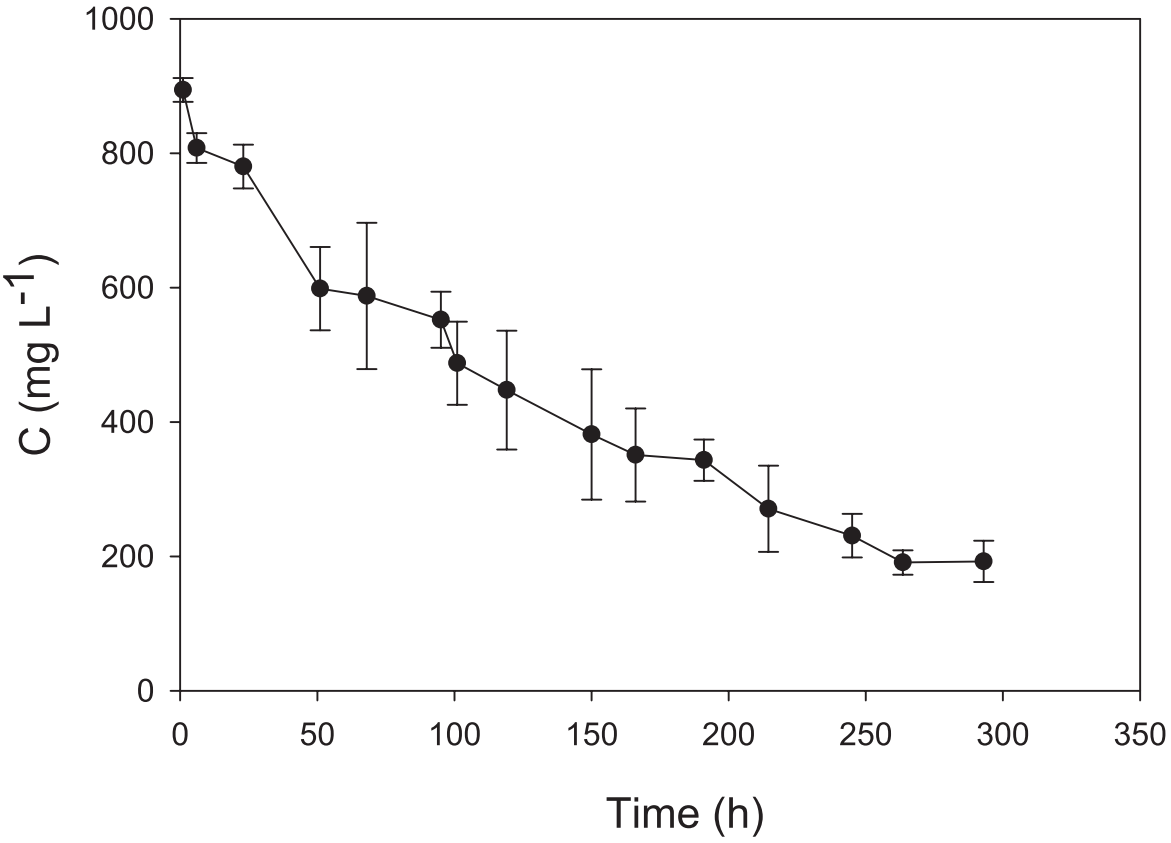
Supplementary Information



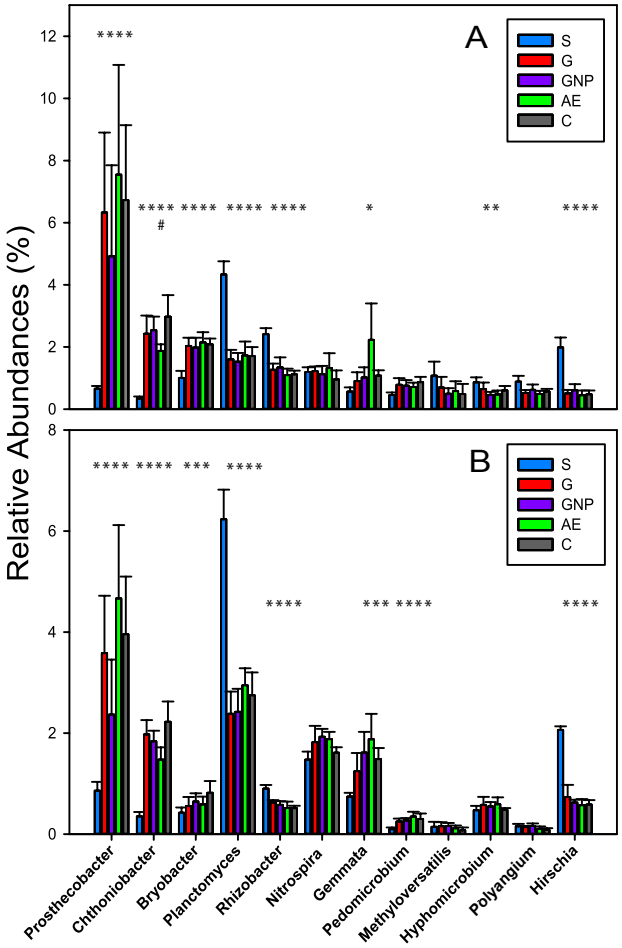


Figure A2. Taxonomic affiliation of the active (A) and the bulk community (B) composition from the different treatments on genus level. Treatments are marked as follows: S-Starter Community, G-GLC, GNP-GLC+N+P, AE-Algae, C-Control. An asterisk indicates significant differences ($p>0.05$) between the starter community and the treatments (G, GNP, AE, C) and a hash indicates significant differences ($p>0.05$) between the control and the treatments (G, GNP, AE).

5. Paper II

Light availability affects stream biofilm bacterial community composition and function, but not diversity

Wagner K, Besemer K, Burns NR, Battin TJ, and Bengtsson MM. (2015). Environmental Microbiology doi:10.1111/1462-2920.12913

My contribution to this study was designing the microcosm set up, processing samples in the laboratory and generating and analysing the 454-pyrosequencing data in close collaboration with Mia Bengtsson. Additionally, I interpreted the results and wrote the manuscript as first author.

environmental microbiology

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Light availability affects stream biofilm bacterial community composition and function, but not diversity

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Summary

Changes in riparian vegetation or water turbidity and browning in streams alter the local light regime with potential implications for stream biofilms and ecosystem functioning. We experimented with biofilms in microcosms grown under a gradient of light intensities (range: 5–152 μmole photons s^{-1} m^{-2}) and combined 454-pyrosequencing and enzymatic activity assays to evaluate the effects of light on biofilm structure and function. We observed a shift in bacterial community composition along the light gradient, whereas there was no apparent change in alpha diversity. Multifunctionality, based on extracellular enzymes, was highest under high light conditions and decoupled from bacterial diversity. Phenol oxidase activity, involved in the degradation of polyphenolic compounds, was twice as high on average under the lowest compared with the highest light condition. This suggests a shift in reliance of microbial heterotrophs on biofilm phototroph-derived organic matter under high light availability to more complex organic matter under low light. Furthermore, extracellular enzyme activities correlated with nutrient cycling and community respiration, supporting the link between biofilm structure–function and biogeochemical fluxes in streams. Our findings demonstrate that changes in light availability are likely to have significant impacts on biofilm structure and function, potentially affecting stream ecosystem processes.

Introduction

Benthic ecosystems are of global significance for biogeochemistry and biodiversity (Covich *et al.*, 2004; Findlay and Battin, in press). In streams, the benthic zone is a thin but critical layer that connects surface with subsurface habitats, including the hyporheic zone and groundwater. Key stream ecosystem processes including nutrient cycling, primary production and respiration are linked to the benthic zone. Benthic biofilms dominate the microbial life in streams (Geesey *et al.*, 1978) and carry out fundamental ecosystem processes such as primary production and organic matter processing (Romani and Sabater, 1999; Battin *et al.*, 2003a; 2008). The study of the algal component in benthic biofilms, traditionally termed periphyton, has been a mainstay in stream ecology over the last decades (e.g. Hill *et al.*, 2003). It is well established that the physical structure and composition of algal communities depend on the flow and light regime (Hill and Boston, 1991; Wellnitz and Rader, 2003). Benthic algae and cyanobacteria are the most important primary producers in streams (Lamberti and Steinman, 1997) and form the trophic basis for invertebrate grazers in streams and are therefore key for carbon transfer (Power *et al.*, 1985).

The appreciation of the prominent role of heterotrophic bacteria in benthic biofilms for ecological and biogeochemical processes in streams is more recent (Findlay, 2010). Given the close spatial proximity of heterotrophic microorganisms and phototrophs such as algae in biofilms, algal-bacterial interactions have received some attention (e.g., Romani and Sabater, 1999; Rier and Stevenson, 2002; Ylla *et al.*, 2009; Rier *et al.*, 2014). It has been shown, for instance, that algal–bacterial interactions are pronounced when algal exudates are abundant during periods of high light availability and photosynthesis, but low when external substrates satisfy the heterotrophic carbon demand in biofilms (Rier and Stevenson, 2002; Ylla *et al.*, 2009). It has also been purported that the interaction between algae and microbial heterotrophs in biofilms could induce priming of putatively recalcitrant dissolved organic matter (DOM) from

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the terrestrial milieu (Bengtsson *et al*., 2014; Rier *et al*., 2014); evidence remains weak, however. Although algal–bacterial interaction in benthic biofilms are likely critical for carbon fluxes in streams (Rier and Stevenson, 2002; Ylla *et al*., 2009), there is little information on how bacterial community composition and function respond to variation in primary productivity, for example due to varying light availability in streams.

The natural light regime in streams is increasingly becoming perturbed. For instance, the riparian deforestation causes high levels of photosynthetically active radiation (PAR) and ultraviolet radiation to reach the streambed, with consequences for algal biomass, primary production and nutrient cycling (e.g. Sweeney *et al*., 2004; Richardson and Béraud, 2014). Furthermore, 'browning' as induced not only by increased terrestrial deliveries of humics, but also elevated turbidity because of increasing erosion may attenuate light intensity in freshwater ecosystems (Karlsson *et al*., 2009). Finally, emerging nighttime light pollution may increasingly impact stream ecosystems (Perkin *et al*., 2014). It is therefore critical to understand the effect of light on microbial biofilms in streams beyond the mere effects that light has on algae.

In this study we experimented with benthic biofilms in microcosms under a gradient of six different light intensities to establish how light impacts bacterial community composition, diversity and community function. While it has been established that light intensity affects algal biomass and activity in biofilms (Adlboller, 2013; Ceola *et al*., 2013), we hypothesized that light also affects bacterial community composition as modulated by phototrophic biomass and primary productivity. This is based on the observation that microbial heterotrophs in biofilms rely more on allochthonous carbon sources (i.e. terrestrially derived DOM) if supply from biofilm phototrophs is reduced (Battin *et al*., 2003b; Ylla *et al*., 2009), which potentially selects for a different bacterial community. At intermediate light intensities, we hypothesized that biofilms may rely to a similar extent on autochthonous DOM sources (i.e. from biofilm phototrophs) and allochthonous DOM sources. Therefore, we expected to observe a peak in bacterial alpha diversity (richness and evenness of operational taxonomic units, OTUs) under these circumstances due to increased resource diversity. We further anticipated a shift in community function along the light gradient, reflecting the decreased reliance of bacteria on phototroph exudates under low light availability. We used 454-pyrosequencing of the 16S rRNA gene to address biofilm bacterial community composition and diversity in combination with extracellular enzyme activity assays to study community function. Furthermore, a multifunctionality index was calculated from the individual enzyme activities to study the effects of biodiversity on multiple ecosystem functions, which is important to avoid the overestimation of functional redundancy in a given ecosystem (Gamfeldt *et al*., 2008; Peter *et al*., 2011). Our focus on bacterial community composition, biodiversity and multifunctionality expands existing knowledge on the relationship between light and biofilms (e.g. Rier and Stevenson, 2002; Lear *et al*., 2008; Rier *et al*., 2014) and sheds new light on community structure and function in stream biofilms.

Results

Influence of light on biofilm biomass and activity

We grew benthic biofilms over a total of 27 days in streamside flumes under 6 different light intensities (generated by shading foils) with 92%, 69%, 51%, 24%, 14% and 7% transmission of the incident light (henceforward termed 92%T, 69%T, 51%T, 24%T, 14%T and 7%T light treatment respectively). This yielded mature biofilms with a biomass ranging from 0.04 to 0.51 mg C cm^{-2} that we gently transferred into laboratory microcosms covered with the same shading foils as during the initial growth phase. Higher light generally resulted in an increase in biofilm biomass, chlorophyll *a* content and bacterial cell counts (first measured on day 1 directly after the transfer to the microcosms), yet the highest values were found at intermediate light intensities (Table S1); this increase was more pronounced after 7 days of experimental work in the microcosms (day 7). These time points were chosen to be representative of the experimental period, representing conditions shortly after the transfer of the biofilms from the field (day 1) and after acclimatization to laboratory conditions (day 7). Inevitably, the laboratory conditions differed from the field conditions, which in part explains the differences observed between time points for most measured parameters (see SI methods for a summary). These temporal changes are not the focus of this study, instead effects attributable to the light gradient observed for both time points are henceforth reported. The light gradient yielded biofilms with varying phototrophic biomass, which resulted in a clear gradient of primary productivity (day 1: $r^2 = 0.70$, $P < 0.001$, day 7: $r^2 = 0.92$, $P < 0.001$), with saturating relationships for gross primary production (GPP) (Fig. 1A). Also, community respiration (R) significantly increased along the light gradient (day 1: $r^2 = 0.75$, $P < 0.001$, day 7: $r^2 = 0.94$, $P < 0.001$) (Fig. 1B). Net primary production (NPP) was always positive throughout the experiment, even under low light availability (Table S1). A ratio of NPP to R of 2.0 ± 0.7 (mean ± SD) in the 7%T light treatment indicates high primary productivity and net-autotrophy even under low light conditions. There was also a net release of dissolved organic carbon (DOC) from the biofilms, which significantly increased with light availability (day 1: $r^2 = 0.24$, $P < 0.05$, day 7: $r^2 = 0.82$,

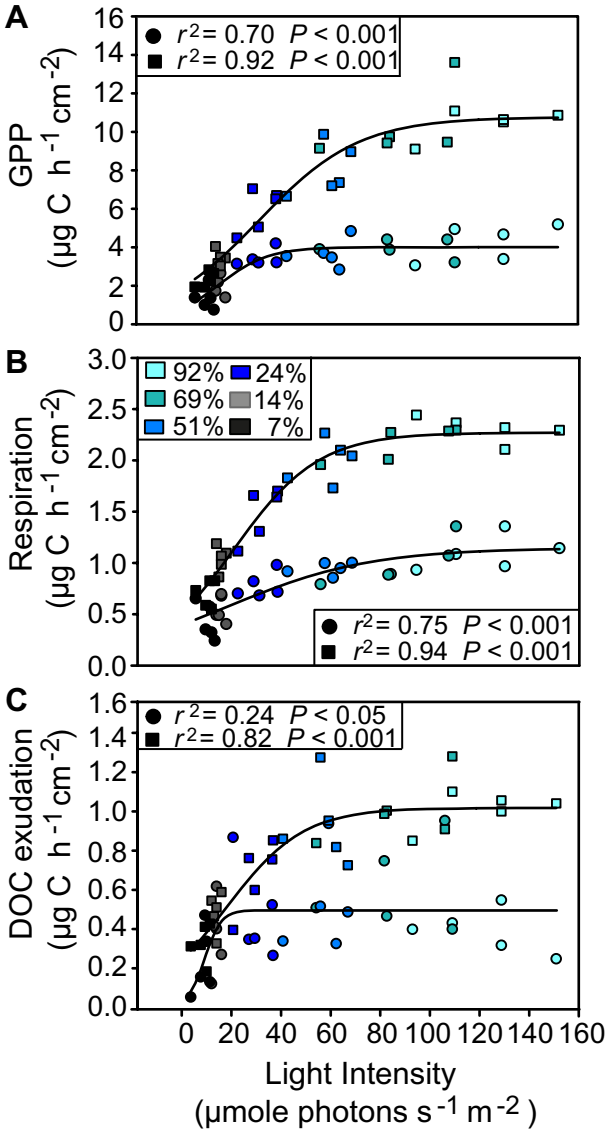

Fig. 1. Non-linear (hyperbolic) regression between GPP (A) respiration (B) and DOC exudation (C), respectively, and the measured light intensities for each microcosm at day 1 (circles) and at day 7 (squares) of the experiment; same colours indicate light treatments (relative transmission (%T) of the incident light).

$P < 0.001$) and probably reflects exudation by biofilm phototrophs (Fig. 1C). However, the presumed simultaneous uptake of DOC by heterotrophs could not be quantified in this study and likely conceals some of the DOC exuded by phototrophs.

Influence of light on biofilm community composition, diversity and multifunctionality

454-pyrosequencing of the 16S rRNA gene and taxonomic classification using classification resources for environmental sequence tags (CREST; Lanzén *et al.*, 2012) revealed clear effects of light on the relative abundance of the phototrophic taxa of the biofilms. The effect of light was particularly evident for *Cyanobacteria*, which showed higher relative abundance under high light conditions both at day 1 and at day 7 of the experiment (Fig. 2A and B). *Cyanobacteria* were the second most abundant taxon and showed significant increases with GPP ($r_s = 0.72$, $P < 0.001$), whereas plastids showed a significantly decreasing relationship with GPP ($r_s = -0.60$, $P < 0.001$). Impacts of the light gradient on the bacterial community were evident on both phylum and genus levels (Fig. 2). Taxa that showed a higher relative abundance under high light conditions included the alphaproteobacterial genera *Roseomonas*, *Rhodobacter* and *Roseococcus*, the betaproteobacterial genera *Polaromonas* and *Rivibacter* (Fig. 2C and D), as well as unidentified taxa belonging to the candidate division TM7. The opposite trend was found within the *Planctomycetes* and *Gemmatimonadetes* (Fig. 2A and B), which were more abundant under low light conditions at day 7. Biofilm community composition also shifted from day 1 to day 7 of the experiment. The relative abundance of the betaproteobacterial genus *Rivibacter* increased significantly during the experiment, while the relative abundance of *Bacillariophyta* (Plastids) and the genus *Flavobacterium* (Bacteroidetes) decreased (Fig. 2).

To address the indirect effect of light on bacterial community composition modulated by phototrophic activity we performed non-metric multidimensional scaling (nMDS) ordination excluding OTUs clustered at a 97% identity level (97% OTUs) identified as *Cyanobacteria* and algal plastids. The nMDS ordination showed an apparent gradient in community composition in response to light and a clear separation of the bacterial community composition from day 1 to day 7 of the experiment (Fig. 3A). Light (measured light intensities) explained 7% (PERMANOVA: $R^2 = 0.07$, $P < 0.01$), and time explained 11% of the variance in community composition (PERMANOVA: $R^2 = 0.11$, $P < 0.01$). GPP, a proxy for algal biomass and activity, explained 10% of the variance in community composition (PERMANOVA: $R^2 = 0.10$, $P < 0.01$). GPP was also a significant predictor for bacterial community composition when both time points were tested separately (Fig. 3A) and showed a significant correlation with the first axis of the nMDS ordination (Fig. 3B).

Richness, evenness, the Simpson as well as the Shannon number equivalents (calculated based on 97% OTU data) were highest in the 24%T light treatment at day 1 of the experiment (Table S2). However, at day 7 of the experiment no significant differences could be observed in any of the alpha diversity measures. No correlation could be detected between richness (day 1: $r_s = 0.13$ $P > 0.05$; day 7: $r_s = -0.11$ $P > 0.05$) and evenness (day 1: $r_s = 0.17$ $P > 0.05$; day 7: $r_s = -0.03$ $P > 0.05$),

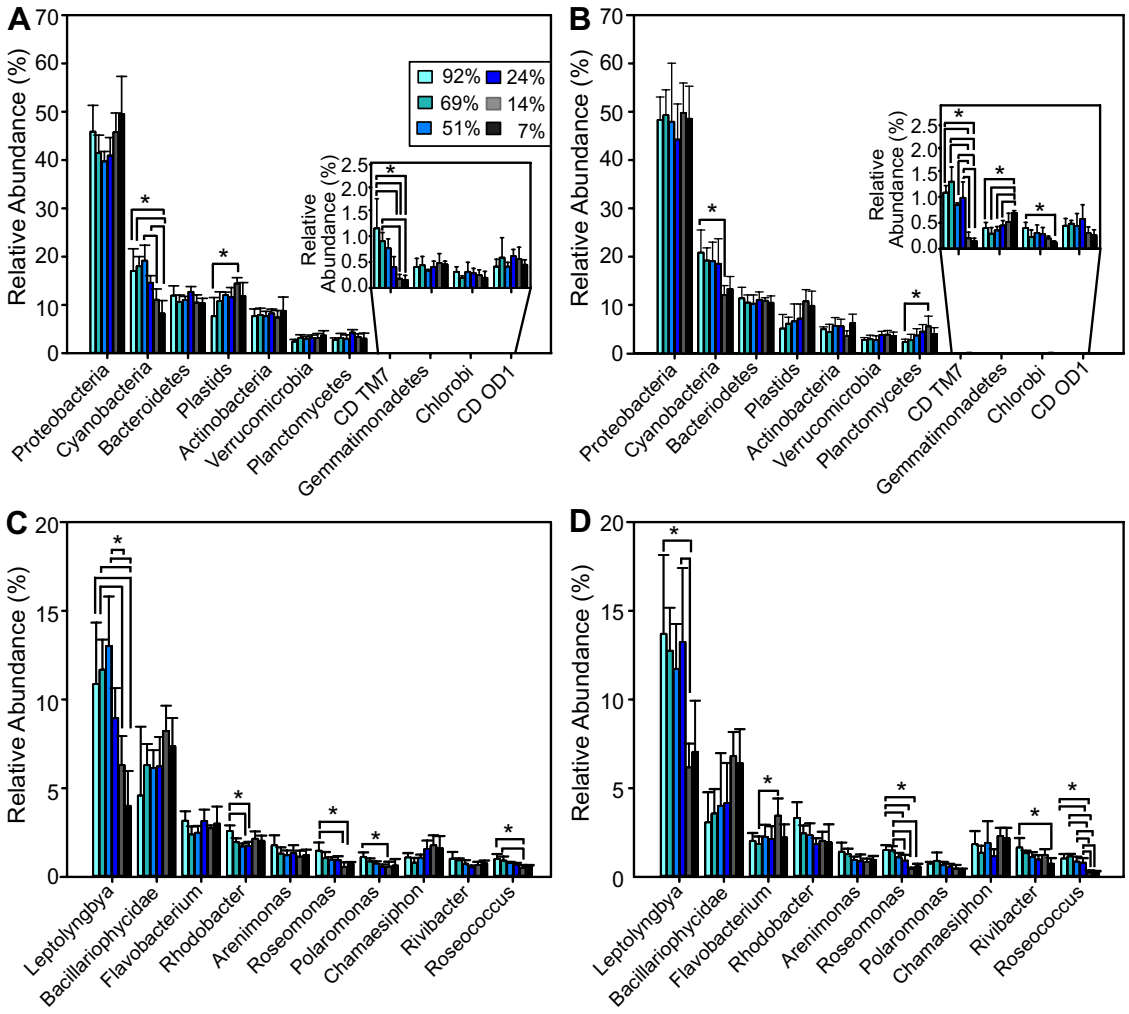

Fig. 2. Relative abundance of taxa based on 454-pyrosequenicng of the 16S rRNA gene at the phylum level at day 1 (A) and at day 7 (B) of the experiment and at the genus level at day 1 (C) and at day 7 (D) of the experiment. The most abundant taxa are displayed on each level. Results are based on taxonomic classification of 97% OTUs. An asterisk (*) indicates significant differences (ANOVA, $P < 0.05$) between light treatments (relative transmission (%T) of the incident light) connected with brackets. Error bars indicate ± 1 standard deviation of the mean based on five replicate samples.

respectively, and GPP at day 1 and day 7 of the experiment. Also community respiration showed no significant correlation with species richness (day 1: $r_s = -0.12$, $P > 0.05$, day 7: $r_s = -0.21$, $P > 0.05$ of the experiment).

A multifunctionality index was calculated from the area-specific extracellular enzymatic activities according to Gamfeldt *et al*. (2008). The probabilities to sustain multifunctionality (multifunctionality index) were significantly higher in the 92%T and the 51%T light treatments than in the 7%T light treatment at day 1 and at day 7 of the experiment (Fig. 5A and B). Interestingly, multifunctionality increased in the 92%T and the 7%T light treatments, while it decreased in the 51%T light treatment from day 1 to day 7 of the experiment. There was no significant correlation between multifunctionality and 97% OTU richness neither at day 1 nor at day 7 (day 1: $r_s = 0.17$ $P > 0.05$, day 7: $r_s = 0.17$ $P > 0.05$).

Influence of light on extracellular enzyme activities, DOC and nutrient dynamics

Phosphatase, leucine-aminopeptidase and beta-glucosidase activity generally increased with light intensity, whereas phenol oxidase activity decreased from the highest to the lowest light treatment at day 7 (Fig. 4). Beta-glucosidase activity significantly correlated with community respiration, showing an increase with light availability, whereas phenol oxidase activity showed a decreasing relationship with community respiration (Fig. 6A and B). Phosphatase activity significantly

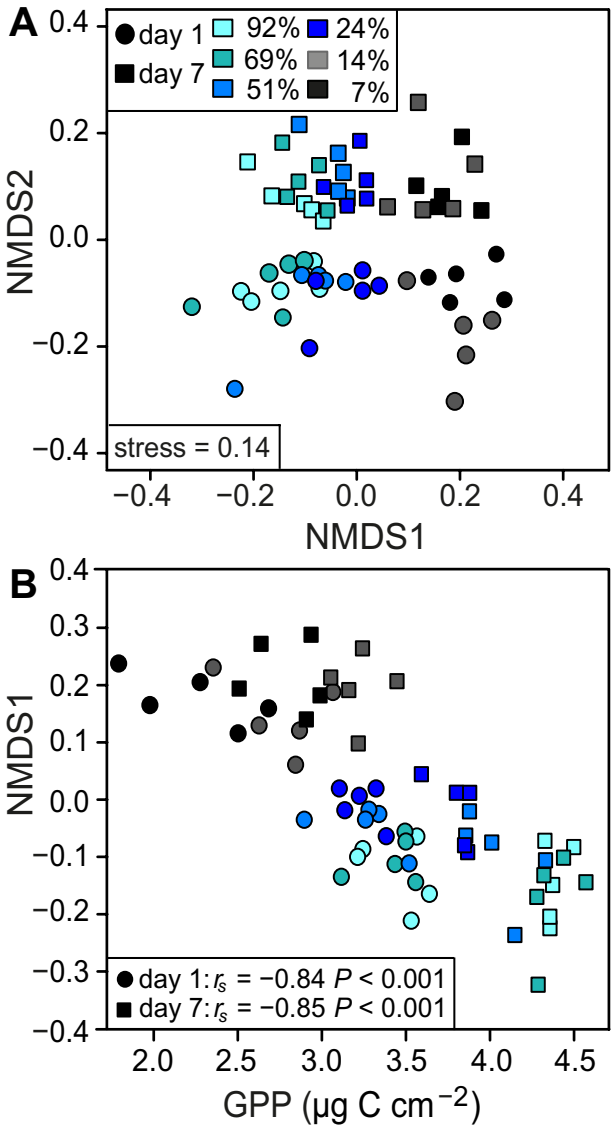

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis distance of the bacterial community composition (97% OTU relative abundances) (A). Spearman's rank correlation between the first axis of the NMDS ordination and GPP (log transformed) (B). Colours indicate light treatments (relative transmission (%T) of the incident light).

correlated with bulk PO_4 uptake and leucine-aminopeptidase activity correlated with NO_3 uptake from the water (Fig. 6C and D). Mantel's test showed a significant correlation between the extracellular enzyme activities and community composition (day 1: $r = 0.52$, $P < 0.001$, day 7: $r = 0.44$, $P < 0.001$).

Discussion

Biofilm community composition, diversity and multifunctionality

We observed a clear shift in bacterial community composition along the light gradient. This supports our hypothesis that light not only affects phototrophic biomass and activity, but also bacterial community composition. There was a direct effect of light on the phototrophic components of the biofilms such as *Cyanobacteria* and on other likely phototrophic bacterial taxa such as *Rhodobacter* and *Roseococcus* (Yurkov, 2006). In addition to these direct effects, some bacterial taxa with predominantly heterotrophic lifestyles appeared to increase with light (e.g. Candidate division TM7, *Roseomonas* and *Rivibacter*), whereas others decreased (e.g. *Planctomycetes* and *Gemmatimonadetes*). This may reflect preferences towards utilization of autochthonous and allochthonous organic matter, respectively, although it is difficult to make functional predictions based on such trends alone. However, the ordination based on 97% OTUs (excluding Cyanobacterial and plastid OTUs) shows a distinct shift along the light gradient which indicates that the community shift is driven by changes at 97% OTU level, and not changes on phylum or genus level. We chose to not address algal community composition in this study, as pervious work on biofilms from the same watershed showed that light primarily affected algal biomass, not species composition (Adlboller, 2013). It is, however, possible that a shift in the algal community could have contributed to the bacterial community shift due to specific associations between algae and bacteria.

While light availability had a clear influence on bacterial community composition, alpha diversity (e.g. 97% OTU richness and evenness) did not show any obvious trends along the light gradient nor did it correlate with any process (e.g. GPP and community respiration) measured. This is remarkable, because thicker biofilms forming under high light availability could be expected to be structurally more complex, therefore including more niches and ultimately elevated diversity (Jackson *et al.*, 2001). Furthermore, according to the classical notion that energy supply limits diversity (Currie, 1991; Cardinale *et al.*, 2009), higher primary production under high light conditions may stimulate diversity. However, we observed no relationship between alpha diversity measures of the bacterial community and GPP in biofilms. The lack of a relationship between alpha diversity and community respiration contrasts findings from simple experimentally assembled communities (Bell *et al.*, 2005). However, it is consistent with the observation that diversity does not scale with 'broad' processes (*sensu* Schimel and Schaeffer, 2012) related to organic matter degradation in soils. Furthermore, it has been proposed that the response of ecosystem processes to increasing microbial diversity saturates as one moves from synthetic assemblages with relatively low diversity to complex and highly diverse natural assemblages (Bell *et al.*, 2009). We anticipated that mixing of taxa involved in the degradation of either autochthonous phototroph-derived DOM or

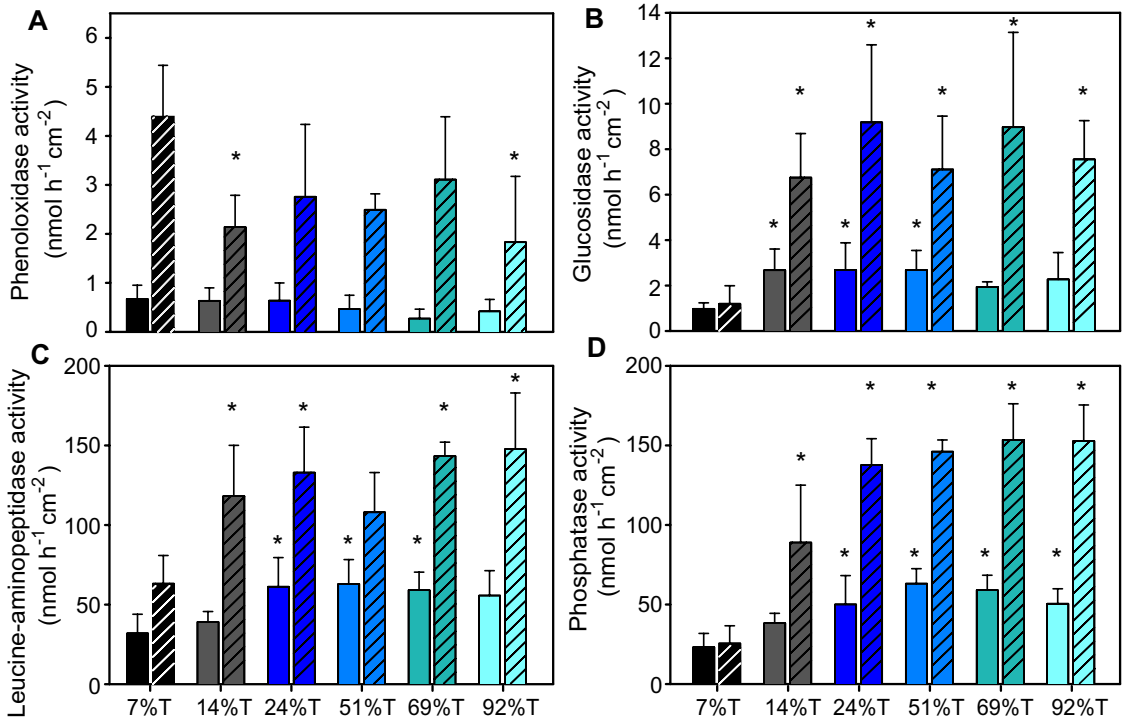

Fig. 4. The areal activity rates of the extracellular enzymes phenoloxidase (A), beta-glucosidase (B), leucine-aminopeptidase (C) and phosphatase (D). Solid bars indicate results from day 1, and shaded bars from day 7 of the experiment. Error bars indicate 1 standard deviation of the mean based on 5 replicate samples. An asterisk (*) above the bars indicates a significant difference (ANOVA, $P < 0.05$) between the 7% light transmission treatment (7%T, lowest light treatment) and the respective treatment within each time point.

allochthonous DOM would lead to elevated diversity in the treatments with intermediate light availability. Indeed, highest microbial diversity was found in the treatment with 24% transmission of the incident light at day 1 of the experiment, which supports our initial hypothesis that higher resource diversity may lead to elevated microbial diversity. However, this effect was not detectable at day 7 of the experiment, indicative that the community shift from day 1 to day 7 of the experiment or other yet unknown factors were more important.

Multifunctionality can be a useful way to aggregate responses of individual processes particularly if these are

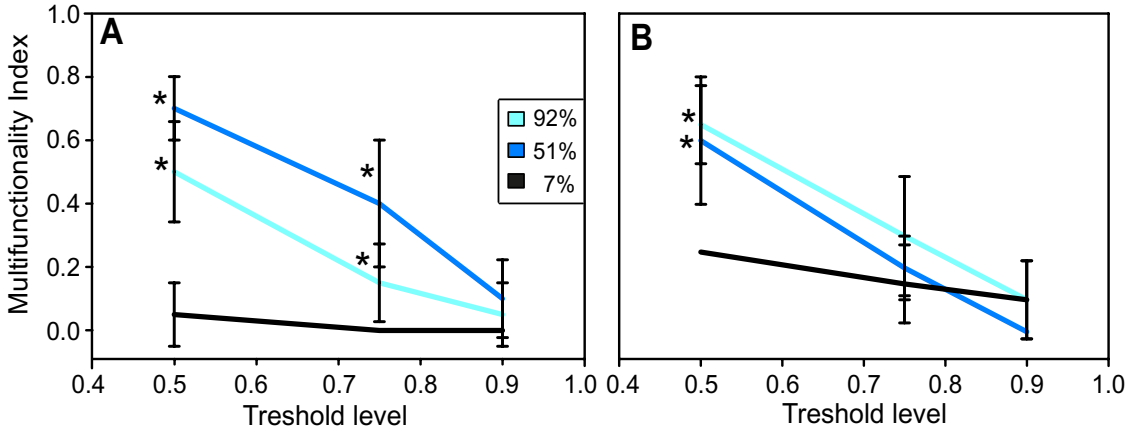

Fig. 5. Probabilities that microbial communities of the treatments (selected to improve clarity of presentation) with 92%, 51% and 7% transmission (%T) of the incident light sustain multifunctionality under different threshold levels at day 1 (A) and at day 7 (B) of the experiment. An asterisk (*) indicates significant differences (ANOVA, $P < 0.05$) between the 7%T and the 92%T and 51%T light treatments respectively.

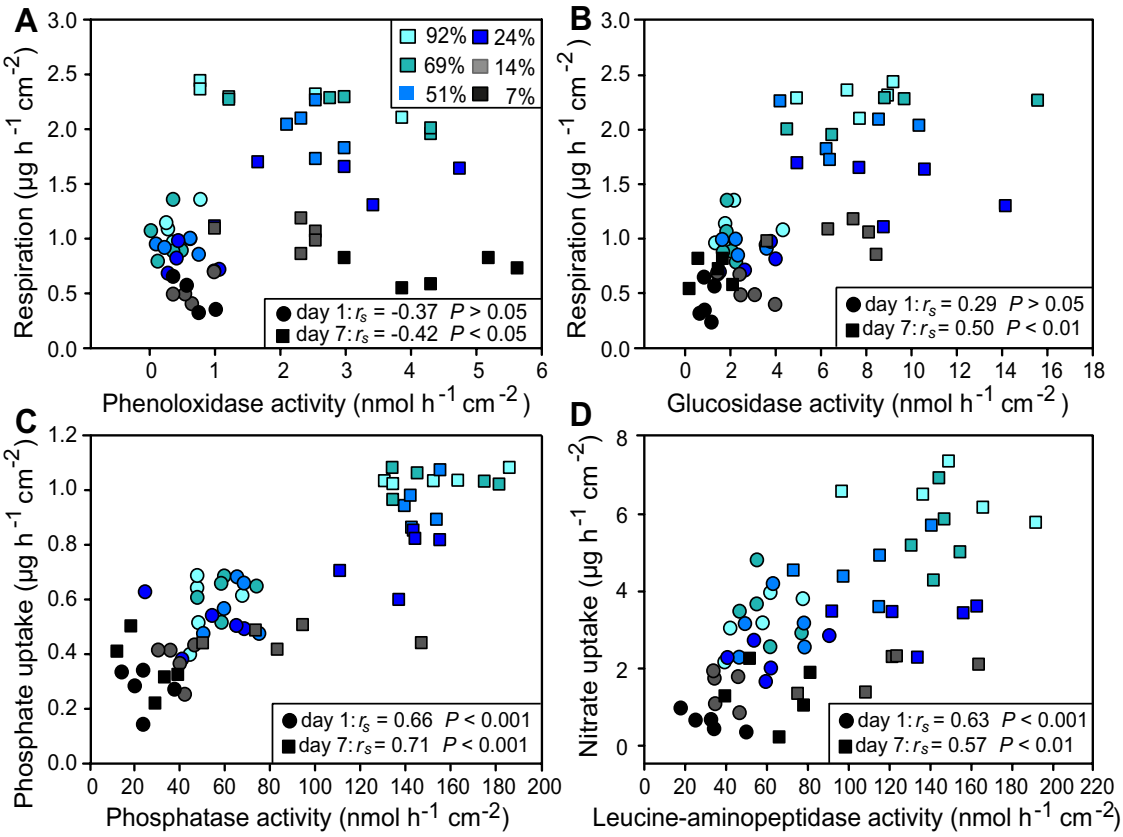

Fig. 6. Spearman's rank correlations of extracellular enzyme activities involved in carbon cycling with respiration (A and B) and enzyme activities involved in P- and N-cycling with PO_4 uptake (C) and NO_3 uptake (D) at day 1 (circles) and at day 7 (squares) of the experiment. Colours indicate light treatments (relative transmission (%T) of the incident light).

'narrow' functions (*sensu* Schimel and Schaeffer, 2012) such as extracellular enzymes expressed to assure a specific physiological pathway or by a phylogenetically constrained microbial group. Our results suggest that multifunctionality as derived from extracellular enzymatic activity was related to light, with highest probability of sustaining multiple enzymatic activities under high light conditions. We propose that the mixture of autochthonous and allochthonous DOM present under high and intermediate light conditions fostered higher community multifunctionality than the allochthonous DOM predominantly available under low light conditions. This finding is consistent with a report on enzyme activity in biofilms showing that resource complexity influenced multifunctionality (Peter *et al.*, 2011).

Phototroph–heterotroph interactions in biofilms

Positive phototroph–heterotroph interactions in biofilms, such as the release of extracellular organic molecules by phototrophs (mainly algae and *Cyanobacteria*) with increasing light intensity (Wood *et al.*, 1992) or the supply of a large specific surface area for the attachment of bacteria (Rier and Stevenson, 2002), sustain heterotrophic metabolisms in stream biofilms (Ylla *et al.*, 2009). We expected to find enhanced phototroph–heterotroph interactions with increasing light intensity, indicated by a parallel increase in abundance of biofilm phototrophs and heterotrophs with light availability. Indeed, we observed higher bacterial cell counts under high light conditions, which were aligned not only with an increase in biofilm biomass and chlorophyll *a* concentration, but also with 'broad' processes such as GPP and DOC exudation, insinuating a positive relationship establishing between biofilm phototrophs and heterotrophs as light intensity increases.

The increased expression of phosphatase, leucine-aminopeptidase and beta-glucosidase with light availability indicates enhanced degradation of phototroph exudates, including peptides and simple polysaccharides, and is further evidence for stronger phototroph–heterotroph interactions under high light conditions (Jones and Lock, 1993; Espeland *et al.*, 2001; Ylla *et al.*, 2009). This corroborates earlier observations on increased expression of leucine-aminopeptidase and beta-glucosidase with phototrophic activity in biofilms

(Haack and McFeters, 1982; Romani *et al*., 2004). In contrast, under low light availability, biofilms were visibly thinner, developed lower biomass and showed higher phenol oxidase expression than under high light conditions. Phenol oxidase is involved in the degradation of lignin and other polyphenolic compounds (Sinsabaugh, 2010) and might therefore be regarded as an indicator for the degradation of more complex allochthonous DOM constituents. This result contrasts with the notion of 'priming', where labile algal exudates stimulate the degradation of allochthonous DOM (Guenet *et al*., 2010). In fact, 'priming' would have resulted in higher phenol oxidase activity under high light conditions. Instead, stronger reliance on allochthonous DOM in thin biofilms under low light availability may be attributable to mass transfer phenomena and decreased phototroph–heterotroph interactions. This notion is in fact supported by earlier observations suggesting enhanced mass transfer of DOM from the bulk liquid to and into thin biofilms to satisfy the carbon demand of the microbial heterotrophs (Battin *et al*., 2003b; Romani *et al*., 2004).

Further evidence towards decreased phototroph–heterotroph interactions is provided by increasing ratios of NPP to respiration with light intensity, shifting community metabolism from relatively more heterotrophic under low light conditions to more autotrophic under high light availability. The bacterial community shift from low to high light availability in combination with increased phenol oxidase activity is indicative of a community-level response to more complex allochthonous carbon sources. Phenol oxidase is assumed to be expressed by a restricted number of microbial taxa (Woo *et al*., 2014). The correlation between community composition and function supports this observation. Altogether, our results are consistent with the view of a biofilm bacterial community that is structured by phototrophs through phototroph–heterotroph interactions under high light, and by allochthonous carbon sources under low light.

Possible implications for stream ecosystem processes

Our study paves the way towards a better mechanistic understanding of the fine-scale processes in biofilms that likely affect ecosystem processes as environmental changes such as riparian deforestation, elevated browning or water turbidity alter the light regime in streams. Our findings suggest that light intensity not only affects overall phototrophic biomass and production in benthic biofilms, but also has direct and indirect cascading effects on the biofilm heterotrophs. For instance, we observed correlations between extracellular enzymatic activity, nutrient cycling and community respiration respectively. We do acknowledge that these correlations may be partly driven by biomass accrual along the light gradient. Nevertheless, these patterns also support the notion of a link between biofilm structure–function and biogeochemical fluxes in streams. Under high light availability, phototrophs likely provide heterotrophs with organic nitrogen and phosphorous, while satisfying their own nutrient demand from inorganic nutrients in the streamwater. It is remarkable that despite lower biomass, biofilms grown under low light availability appeared to be more efficient in degrading allochthonous DOM as indicated by the higher phenol oxidase activity. Thus, stream reaches that experience limited light, for example, due to intact riparian vegetation, may process more allochthonous DOM, which has important consequences for landscape level carbon cycling.

Our findings suggest that microbial diversity apparently remains decoupled from biofilm production as light availability changes. However, the source of the available substrates (i.e. from phototroph exudates or of terrestrial origin) may have modulated microbial diversity along the light gradient. Elevated interactions between biofilm phototrophs and heterotrophs with increasing light availability are also supported by our findings on enzymatic activity. Collectively, our findings therefore evoke that as light becomes attenuated in streams, biofilms rely more on DOM constituents from the streamwater with no apparent impact on microbial diversity but with clear shifts in bacterial community composition and function.

Experimental procedures

Microcosm setup and sampling design

Benthic biofilms were established for a total of 27 days on glass slides (1 cm^2) in streamside flumes fed with raw streamwater from Oberer Seebach (OSB, Lunz am See, Austria, 600 m above sea level) in June 2012 (see SI Methods for further information on OSB). After 18 days of establishment, all slides were brushed with a soft brush to remove loosely attached biomass, and biofilms were then allowed to regrow during 9 days. Based on previous experience (Tom Battin, unpublished), this method yields uniformly compact biofilms that do not easily disintegrate during handling. To achieve a gradient in phototrophic biomass, slides were covered with neutral-density photographic filters (LEE filters, Burbank, California, USA) during biofilm establishment, generating a light gradient with six levels. These filters attenuate incident light but do not change the spectral distribution and have been previously used for stream biofilm growth (Ceola *et al*., 2013). We used filters 226, 298, 209, 210, 211 and 299 to yield transmission of 92%, 69%, 51%, 24%, 14% and 7% of the incident PAR respectively. Ninety slides were then transferred into each air-tight Plexiglas© microcosms covered with the same light filters. Each light treatment was replicated in five microcosms. All 30 microcosms were randomly distributed under a fluorescent light source (Phillips, TL-D 58W/33) with a 14:10 h day : night regime. PAR intensity was measured inside each microcosm (LI-COR 1400, WALZ US-SQS/L sensor). Microcosms had magnetic stirring to ensure continuous mixing of water

overlying biofilms. Temperature was measured inside selected microcosms and averaged 18.0 ± 0.9°C (mean ± SD) and 16.7 ± 0.5°C during day and night respectively.

Biofilms in the microcosms were incubated with oligotrophic groundwater from a nearby spring, as it is similar to the groundwater that feeds OSB at baseflow. Water in each microcosm was replaced twice daily and spiked with a cold-water extract from crack willow that served as allochthonous DOM (Wagner *et al.*, 2014). This groundwater–willow extract mixture is henceforward referred to as feed water. We collected water samples for the analyses of DOC, P-PO_4 and N-NO_3 concentration from the feed water and after recirculation (7 h) from the microcosms. Concentrations of DOC, P-PO_4 and N-NO_3 in the feed water were 0.879 ± 0.053 mg C l^{-1}, 69.3 ± 3.8 μg P-PO_4 l^{-1} and 1208 ± 17.2 μg N-NO_3 l^{-1} respectively. Replicate biofilm samples for 454-pyrosequencing (3 slides per microcosm), enzyme activity analysis (1 slide per microcosm and enzyme), microbial cell counts (6 slides per microcosm) and the determination of chlorophyll *a* concentration (6 slides per microcosm) were randomly collected from the microcosms at day 1, several hours after the transfer of the biofilms to the microcosms, and at day 7, before the termination of the experiment. These sampling times were chosen to be representative of the experimental period.

Solute removal and metabolism

The areal removal rates *(R)* of DOC, PO_4 and NO_3 were calculated according to $R = (\Delta C * V)/(T * A)$ where ΔC is the difference in concentration between the feed water and the output of the microcosm measured over one recirculation period, *V* is the water volume (0.75 l) in the microcosm, *T* is the recirculation time (in hours), and *A* is the total surface area (in cm^2) of all slides present in the microcosm at a given time.

Concentration of dissolved oxygen (DO) was measured at the beginning and at the end of each recirculation period using planar optodes (PSt3 sensor, Presens, Germany). DO production (during day) and DO consumption (during night) were used to infer NPP, R and GPP (Bott, 1983). We assumed a respiration quotient of 0.81 as used previously for freshwater ecosystems (Berggren *et al.*, 2011; Bengtsson *et al.*, 2014). N-NO_3 and P-PO_4 concentrations were determined
on a continuous flow analyser (FlowSys 3rd generation, SYSTEA Analytical Technologies) on sterile filtered (0.2 μm) samples; DOC concentrations in filtered (Whatmann GFF) samples were measured on a TOC Analyzer (Sievers 5310C, GE Analytical Instruments). All glassware was acid-washed and combusted (450°C, 4 h).

Extracellular enzyme activities

Biofilms were sampled for the analysis of extracellular enzyme activity at day 1 and at day 7 of the experiment. The extracellular enzymes leucine-aminopeptidase (EC 3.4.11.1), phosphatase (EC 3.1.3) and beta-D-glucosidase (EC 3.2.1.21) were selected because of their relevance in C-, N- and P-cycling; their activities were measured spectrofluorometrically using aminomethylcoumarin (AMC) and methylumbelliferyl (MUF) (Sigma Chemical Company). Hydroximethylether was added to the MUF substrates (final solution in the assay of 0.1 %) to facilitate their dissolution in water. The MUF and AMC reference standards were prepared with autoclaved MQ water. Phenol oxidase activity (EC 1.14.18.1) was measured using 3,4-dihydroxyphenylalanine (L-DOPA) (Sinsabaugh, 1994). Saturation curves for each enzyme were made before the start of the experiment to determine enzyme–substrate saturation conditions. One biofilm sample per microcosm was collected at day 1 and at day 7 for each extracellular enzyme analysis ($n = 30$ per enzyme), placed into a pre-combusted glass vial containing 4 ml of sterile filtered (0.2 μM) water from the respective microcosm and the corresponding substrate was added. All assays were conducted under substrate saturation conditions and incubated for 1 h (18°C) on a shaker in the dark to avoid possible photo-degradation of the substrates. For each enzyme, we used negative controls for substrate color (0.2-μm-filtered water from the microcosms and substrate analog) to assess the abiotic degradation of the artificial substrate. At the end of the incubation, glycine buffer (pH = 10.4) was added to the biofilm samples and to the controls. The fluorescence of the MUF-substrates was read at EX365nm and EM455nm, the fluorescence of the AMC-substrates was read at EX364nm and EM445nm (F-7000 Hitachi). Phenol oxidase activity was measured at 450 nm (UV-1700 PharmaSpec, Shimadzu). Enzyme activities were expressed as nmol of substrate converted h^{-1} cm^{-2}.

DNA extraction, PCR and 454-pyrosequencing

Biofilms were sampled for 454-pyrosequencing of the 16S rRNA gene at day 1 and at day 7 of the experiment. Triplicate samples were collected from each microcosm, flash frozen in liquid N_2 and stored at −80°C. Total nucleic acids were extracted from the biofilm (Urich *et al.*, 2008). The bacterial hypervariable regions V3 and V4 of the 16S rRNA gene were amplified with the forward primer 341F (5′-CTACGGGNGGCWGCAG-3′) and the reverse primer 805R (5′-GACTACHVGGGTATCTAATCC-3′) combination (Logue *et al.*, 2012) in a two-step PCR protocol (for detailed information please see SI Methods). PCR products were purified by agarose gel electrophoresis and the QIAquick Gel Extraction Kit (QIAGEN) following the producer's recommendations. The purified PCR products were quantified using the DNA binding QuantiFluor™ dsDNA System Kit (Promega Corporation). Amplicons were pooled in equimolar concentrations to obtain similar numbers of 454-pyrosequencing reads per sample. Amplicons were sequenced on a GS FLX Titanium Sequencer at the Center for Genomic Research (University of Liverpool, UK). 454-Pyrosequencing data were de-noised and reads were clustered at a 97% identity level to operational taxonomic units (97% OTUs) using the software package AmpliconNoise V1.28 (Quince *et al.*, 2011). Taxonomic assignments were determined using CREST (Lanzén *et al.*, 2012).

Microbial cell counts, chlorophyll a *and bulk biomass*

Six biofilm samples were randomly collected from each microcosm at day 1 and at day 7 and conserved in 2.5%

formaldehyde (24 h, 4°C). Prior to cell counting, we added 20 ml pyrophosphate (0.025 mM pyrophospate, 2.5% formaldehyde), and samples were shaken (60 min) and sonicated three times for 20 s (14% amplitude, 1s pulse, 1s pause) to disaggregate cells. Larger particles were allowed to settle from the supernatant for 20 min. Nucleic acids were stained using SYTOX Green (Life Technologies Corporation) (5 μM final concentration, 15 min) and cells were counted on a Cell Lab Quanta SC (Beckman Coulter). An additional six glass slides were sampled from each microcosm at day 1 and at day 7 and chlorophyll *a*, the most important photosynthetic pigment in algae and cyanobacteria and a proxy for phototrophic biomass, was extracted in acetone overnight (4°C). Samples were then vortexed, filtered (Whatmann GFF) and absorbance was measured at 665 nm and 750 nm with a spectrophotometer (UV-1700 PharmaSpec, Shimadzu). Bulk biomass was measured as C content of six biofilm samples using an Elemental Analyzer (EA1110; CE Instruments, Thermo Fisher).

Data analyses

For nMDS ordination, phototrophic- (*Cyanobacteria* and algal plastid), unclassified- and rare 97% OTUs (present in less than 5% of the samples) were excluded. We computed a similarity matrix using the Bray–Curtis index and subjected it to nMDS to visualize the community dynamics of the biofilms. PERMANOVA was used to test the significant effect of light intensity, GPP and time on the bacterial community composition. Alpha diversity was calculated as 97% OTU richness, evenness, the number equivalents of the Shannon and of the Gini–Simpson index from resampled 454-pyrosequencing data, excluding 97% OTUs identified as *Cyanobacteria* and algal plastids (Jost *et al*., 2010; Besemer *et al*., 2013). We used this family of indices as they differently weigh abundant and rare species. All samples were rarefied to the lowest number of reads ($n = 1002$) obtained from a sample prior to analysis. Taxon relative abundance, alpha diversity and biofilm biomass measures were tested for significant differences between light treatments using ANOVA and post hoc Tukey test. We recognize the limitations inherent to comparison of relative abundances, as a change in the relative abundance of a taxon may not reflect a corresponding change in absolute abundances. Nonetheless, relative abundances as are produced by sequencing approaches are frequently used to characterize changes in overall microbial community compositions (e.g. Gilbert *et al*., 2011).

We used the extracellular enzyme activities to infer a multifunctionality index (Gamfeldt *et al*., 2008). Multifunctionality was calculated using the individual extracellular enzyme activities from day 1 and from day 7 of the experiment. If one of the enzyme activities dropped below a predefined threshold (0.5, 0.75, 0.9) of the maximal enzyme activity for this enzyme, the function was considered lost. This means for the 0.5 threshold, for instance, that as long as the community is able to perform 50% of the maximal enzyme activity in all samples, the function is considered retained, whereas if functioning for a specific enzyme dropped below 50% a loss of function was inferred. Spearman's rank correlation was used to test significant correlations between species richness and evenness, respectively, and GPP. All statistical analyses were performed using the software and statistical computing environment R (R Development Core Team, 2014).

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Biofilm parameters from all light treatments (relative transmission (%T) of the incident light) at day 1 and at day 7 of the experiment; given are mean ± SD over the light treatments; analysis of variance (ANOVA) displays significant differences ($P < 0.05$) between light treatments.

Table S2. Alpha diversity of the biofilm community from all light treatments (relative transmission (%T) of the incident light) at day 1 and at day 7 of the experiment; given are mean ± SD over the light treatments; analysis of variance (ANOVA) displays significant differences ($P < 0.05$) between light treatments.

Supplementary Information

Experimental conditions.

Some conditions differed between the outdoor streamside flumes where biofilms were established and the microcosms where they were kept during the experiment. For example, temperature averaged 18.0±0.9 °C during day and 16.7±0.5 °C during night in the microcosms whereas the average temperature in the flume was 9.3±1.6 °C. Light conditions also differed, with a maximum of 152 µmol photons s^{-1} m^{-1} achieved by the fluorescent light source, while outdoor daylight conditions typically range between 150 and 2000 µmol photons s^{-1} m^{-1} in the area (not measured during biofilm establishment). DOC concentrations were of a similar range in the microcosms (feed water mean: 0.879±0.053 mg L^{-1} recirculated mean: 1.14±0.13 mg L^{-1}) compared to the typical ambient concentrations encountered in the OSB. Likewise, NO_3 concentrations were not unusual compared to natural conditions. However, the concentration of PO_4 was higher in the microcosms (feed water mean: 69.3±3.8 µg L-1) than what is typical for the OSB (<10 µg L-1).

Primer specifications and PCR conditions.

First, the universal forward primer 341F (5'-CTACGGGNGGCWGCAG-3') and reverse primer 805R (5'-GACTACHVGGGTATCTAATCC-3') (Thermo Fisher Scientific Inc.) were used to amplify the bacterial genomic DNA. Second, the products from the first PCR were amplified with 341F and 805R amended with the 454-Titanium A and B adaptors, respectively. In addition, the forward primer contained unique barcodes. DNA concentrations of the samples were determined (QuantiFluor™ dsDNA System, Promega Corporation) and template input was adjusted to equal concentrations in all PCR reactions. The 20 µl PCR reactions contained each primer without barcodes at 0.4 mmol L^{-1} (Thermo Fisher Scientific Inc.), dNTPS at 0.65 mmol

L^{-1} (Thermo Fisher Scientific Inc.), bovine serum albumin at 40 mg L^{-1} (Thermo Fisher Scientific Inc.), $MgCl_2$ at 2.0 mmol L^{-1} (Thermo Fisher Scientific Inc.) and Phusion High-Fidelity DNA Polymerase (2U μl^{-1}) with the recommended PCR buffer (Thermo Fisher Scientific Inc.).
In the first PCR step, the 16S rRNA gene was amplified using an initial denaturing step at 94°C for 1 min, followed by 25 cycles of denaturation at 94°C for 30s, annealing for 30s (touchdown PCR; starting at 62°C and decreasing to 50°C), elongation at 72°C for 1 min and a final elongation at 72°C for 10 min. Each reaction was run in technical duplicates and subsequently pooled. In the second PCR step, the conditions were modified slightly: Bovine serum albumin was omitted, primers with adaptors and barcodes at concentrations of 0.8 mmol l^{-1} were used. The annealing temperature was fixed at 56°C for 30 s and the PCR was run for only 5 cycles.
The following accession number was obtained upon submission of the sequences: SRX803716.

Table S1. Biofilm parameters from all light treatments (relative transmission (%T) of the incident light) at day 1 and at day 7 of the experiment; given are mean ± SD over the light treatments; analysis of variance (ANOVA) displays significant differences ($P < 0.05$) between light treatments.

		92%T (a)	69%T (b)	51%T (c)	24%T (d)	14%T (e)	7%T (f)	ANOVA
Day 1	Chlorophyll *a* (µg cm^{-2})	2.1±1.0	2.1±0.9	2.4±0.8	3.1±0.8	2.8±0.9	0.9±0.5	d-f, e-f
	Biomass (mg C cm^{-2})	0.37±0.32	0.60±0.55	0.23±0.12	0.29±0.20	0.15±0.11	0.11±0.04	
	Cell count (10^7 cm^{-2})	3.1±1.9	3.8±1.5	4.1±1.9	3.8±1.4	2.7±1.1	1.8±0.5	
	Net primary production (µg C h^{-1} cm^{-2})	3.0±0.8	3.0±0.2	2.6±0.7	2.5±0.3	1.6±0.6	0.9±0.5	a-e, b-e, a-f, b-f, c-f, d-f
Day 7	Chlorophyll *a* (µg cm^{-2})	3.8±0.7	3.7±0.7	3.7±0.4	4.0±0.4	3.2±1.1	2.0±0.5	a-f, b-f, c-f, d-f
	Biomass (mg C cm^{-2})	0.37±0.13	0.42±0.12	0.21±0.03	0.31±0.22	0.13±0.06	0.06±0.03	a-e, a-f, b-e, b-f , d-f
	Cell count (10^7 cm^{-2})	9.0±1.2	10.1±2.6	8.0±2.7	7.9±2.1	6.0±0.9	3.4±1.1	b-e, a-f, b-f, c-f, d-f, e-f
	Net primary production (µg C h^{-1} cm^{-2})	7.9±0.8	7.9±1.7	5.8±1.1	4.3±0.8	2.3±0.3	1.6±0.3	a-c, a-d, a-e, a-f, b-c, b-d, b-e, b-f, c-e, c-f, d-e, d-f

Table S2. Alpha diversity of the biofilm community from all light treatments (relative transmission (%T) of the incident light) at day 1 and at day 7 of the experiment; given are mean ± SD over the light treatments; analysis of variance (ANOVA) displays significant differences ($P < 0.05$) between light treatments.

		92%T (a)	69%T (b)	51%T (c)	24%T (d)	14%T (e)	7%T (f)	ANOVA
Day 1	Richness	414.9±20.3	430.7±23.7	448.8±10.3	476.7±33.6	415.8±31.8	418.5±49.3	a-d, d-e
	Simpson NE	130.7±15.9	138.3±16.8	153.9±6.6	181.8±29.7	121.1±38.3	131.3±28.7	a-d, d-e, d-f
	Shannon NE	240.4±21.7	254.1±23.2	275.5±8.4	309.7±35.6	237.4±41.3	243.9±45.2	a-d, e-d, e-f
	Evenness	0.91±0.01	0.91±0.01	0.92±0.003	0.93±0.01	0.91±0.02	0.91±0.01	d-e
Day 7	Richness	370.6±59.0	361.6±42.5	371.2±64.2	399.1±31.7	346.7±54.5	379.4±43.4	
	Simpson NE	119.3±44.9	103.1±19.6	100.0±52.7	113.8±32.2	90.6±31.6	118.2±28.8	
	Shannon NE	210.8±59.9	196.0±34.3	197.9±71.9	225.9±35.6	183.0±48.3	216.5±42.4	
	Evenness	0.90±0.02	0.89±0.01	0.90±0.02	0.90±0.02	0.89±0.02	0.90±0.02	

6. Paper III

Light intensity mediates a shift from allochthonous to autochthonous carbon use in stream biofilms

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My contribution to this study was designing the microcosm set up, performing the experiment and analysing the ^{13}C data with a concentration-dependent isotope mixing model in close collaboration with Amber Ulseth. Additionally, I interpreted the results and wrote the manuscript as first author.

Light intensity mediates a shift from allochthonous to autochthonous carbon use in stream biofilms

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Running head: Effect of light on stream biofilms

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Abstract.

Deforestation of the riparian vegetation or increased water turbidity and browning alter the local light regime in the benthic zone of streams, which may have important implications for carbon (C) uptake, transformation and long-term C storage by stream biofilm communities. To investigate the influence of light availability on the uptake dynamics of autochthonous and allochthonous dissolved organic carbon (DOC) in benthic biofilms, we used a microcosm approach with a light intensity gradient (ranging from 5 to 152 μmole photons $m^{-2} s^{-1}$) in combination with a ^{13}C-labeled allochthonous DOC source. We calculated a DOC mass balance, which indicated that benthic biofilms constitute C sources across the entire light intensity gradient. Clear diurnal patterns indicated that benthic biofilms were relying more on allochthonous C sources at night, whereas during the day mainly C from autochthonous sources was respired. Furthermore, phenol oxidase activity and allochthonous DOC uptake were increased under low light availability, which suggests an enhanced degradation of refractory substances under low light conditions. In contrast, beta-glucosidase activity increased with light intensity, suggesting enhanced use of autochthonous DOC under high light availability. Collectively our results suggest that biofilms exposed to high light availability preferentially use bioavailable autochthonous DOC sources, whereas this shifts towards more allochthonous sources as light becomes more attenuated.

Introduction.

Microbial biofilms covering the sediment of streambeds are major sites for the uptake, storage and transformation of dissolved organic carbon (DOC) and inorganic nutrients in stream ecosystems (Bott et al. 1984, Battin et al. 2003b, Romaní et al. 2004). The benthic biofilms exposed to light comprise eukaryotic algae and cyanobacteria, which constitute an important pillar of the stream food web and can sustain a large consumer community (Mcintire 1973, Lamberti and Steinman 1997). Algae and cyanobacteria co-occur with heterotrophic bacteria, all of them being encapsulated in a matrix composed of extracellular polymeric substances (EPS). The spatial proximity of these microorganisms makes benthic biofilms particularly active and important for key ecosystem processes. Algal exudates, often containing amino acids and monomeric sugars (Espeland et al. 2001), are readily taken up by heterotrophic bacteria, which in turn provide carbon dioxide (CO_2) to the algae (e.g. Haack and McFeters 1982, Kaplan and Bott 1989, Battin et al. 2003a). A significant fraction of the gross primary production (GPP) in benthic biofilms is respired by the microbial heterotrophs, which makes these communities relevant for whole-stream ecosystem metabolism and nutrient cycling (Battin et al. 2003b). Furthermore, the spatial proximity facilitates the hydrolytic activity of extracellular enzymes, which can make up a notable fraction of the overall microbial activity in benthic biofilms (Romaní and Sabater 1999).

Microbial heterotrophs dwelling in stream biofilms are exposed to a wide array of DOC compounds that vary in source, chemical composition and bioavailability (Seitzinger et al. 2005, Fasching et al. 2014, Mosher et al. 2015). Besides the autochthonous DOC, these microorganisms also metabolize allochthonous DOC that largely derives from terrestrial vascular plants and that is typically more resistant to degradation than autochthonous DOC. However,

terrestrial deliveries of allochthonous DOC into streams and its subsequent metabolism complement in-stream primary production so that most stream ecosystems are net heterotrophic (Battin et al. 2008). This is especially true for headwater streams that are tightly connected to the terrestrial milieu and where primary production is often restricted because of light limitation. It is generally thought that extended residence times of apparently refractory DOC within biofilms facilitate its degradation (Battin et al. 2003b, Bottacin-Busolin et al. 2009). The relative concentration of extracellular enzymes in biofilms relative to the ambient water may also enhance the hydrolysis of complex compounds within the allochthonous DOC pool (Romaní et al. 2004). Furthermore, labile autochthonous DOC moieties may stimulate the degradation of apparent refractory compounds within biofilms, a phenomenon commonly referred to as the priming effect (e.g. Guenet et al. 2010). The priming effect may influence the metabolic fate of autochthonous versus allochthonous DOC, thereby affecting the function of stream biofilms and their involvement in stream C cycling.

Climate change is thought to augment terrestrial DOC deliveries into streams with consequences for ecosystem respiration and CO_2 emissions (Lapierre et al. 2013, Fasching et al. 2014). The humic and fulvic moieties of these terrestrial deliveries may cause the brownification of streams with potential impacts on the light regime and primary production (Roulet and Moore 2006, Karlsson et al. 2009, Hansson et al. 2013). Other factors such as the deforestation of the riparian vegetation (Sweeney et al. 2004), increased sediment loads (Parkhill and Gulliver 2002) and urban nighttime light pollution (Rich 2005) increasingly change the natural light regime in stream ecosystems.

The impacts of light on the composition and physical structure of benthic algal communities has been a mainstay in stream ecology (e.g. Hill and Boston 1991, Wellnitz and Rader 2003, Zippel

and Neu 2005). However, our understanding on the coupling between light, phototrophic and heterotrophic processes, and C cycling in biofilms remains limited at present. Illuminating these processes is relevant because of the involvement of biofilms in critical ecosystem functions of streams and in the fate of DOC.

The aim of this study was to evaluate the relative importance of autochthonous and allochthonous DOC for the metabolism of stream benthic biofilms under different light availabilities. We expected that increasing light availability augments algal biomass and stimulates primary production, respiration, extracellular enzymatic activity and the exudation rate by algae. We also hypothesized that light would modulate C cycling in biofilms by shifting the relative importance of allochthonous versus autochthonous DOC metabolism either due to priming by algal exudates or preferential use of algal exudates over allochthonous DOC. We grew benthic biofilms under controlled light regimes, and incubated them in microcosms with ^{13}C-labelled allochthonous DOC, which in combination with a mixing model served to track C fluxes such as DOC uptake and exudation by algae. We also determined net primary production (NPP), respiration (R), and activities of extracellular enzymes involved in carbon and inorganic nutrient cycling.

Materials and Methods

Microcosm and sampling design — Microcosms designed to study C fluxes in benthic biofilms were constructed and operated as detailed in Wagner et al. (2015). Briefly, we grew benthic biofilms on glass slides (1 cm^2) in streamside-flumes fed with raw streamwater from Oberer Seebach (OSB, Lunz am See, Austria, 600 m above sea level) for 27 days (June 2012). In these flumes, neutral-density LEE filters (Burbank, California USA) generated six levels of light

intensity to yield a gradient of algal biomass; these filters attenuate incident light but do not change the spectral distribution and have been previously used for stream biofilm growth (Ceola et al. 2013). We used filters 226, 298, 209, 210, 211 and 299 to yield transmissions of 92%, 69%, 51%, 24%, 14% and 7% of the incident photosynthetic active radiation (PAR) (henceforward termed 92%T, 69%T, 51%T, 24%T, 14%T and 7%T light treatments). After 27 days of growth in the streamside-flumes, we transferred the slides covered with biofilms into air-tight microcosms (Plexiglas©, n=90 per microcosm) that were covered with the same filters as applied in the streamside-flumes. We used five replicate microcosms for each light treatment. All 30 microcosms were exposed to the same incident light (Phillips, TL-D 58W/33) with a 14:10 hours day:night regime and we measured PAR (LI-COR 1400, WALZ US-SQS/L sensor) inside each microcosm. Magnetic stirrers ensured continuous mixing of water overlying biofilms in the microcosms. Average water temperature was 18.0±0.9°C (mean±SD) during the day and 16.7±0.5°C at night. We incubated the biofilms in artificial streamwater (10.9 ±1.7 µg C cm^{-2}) that we produced from groundwater enriched with a cold-water extract from ^{13}C-labeled willow (*Salix fragilis*, Wagner et al. 2014); this artificial streamwater reflects basic chemical properties of the streamwater in OSB (Table SI 1), which is largely a groundwater-fed stream at baseflow. This artificial streamwater was added to the biofilms and recirculated in the microcosms on average for 6 hours during each day and night. We collected streamwater samples from the microcosms at the beginning (t_{0h}) and at the end (t_{6h}) of each recirculation.

Microbial cell counts, chlorophyll a and bulk biomass — We measured chlorophyll *a* content, bacterial cell abundance and biomass C from random biofilm samples from each microcosm after 8 h and 128 h of the experiment. To estimate cell abundance we detached cells using 20 ml

pyrophosphate (0.025 mM pyrophospate, 2.5% formaldehyde) and sonication, stained cell nucleic acids using SYTOX Green (Life Technologies Corporation) (5 µM final concentration, 15 min) and counted cells on a Cell Lab Quanta SC (Beckman Coulter). Chlorophyll *a* was extracted from biofilms in acetone over-night (4°C) and determined spectrophotometrically (UV-1700 PharmaSpec, Shimadzu) at 665 nm and 750 nm. Bulk biomass was measured as organic C and its ^{13}C content was determined using elemental analysis-isotope ratio mass spectrometry (EA-IRMS on an EA1110; CE Instruments, Thermo Fisher, Department of Terrestrial Ecosystem Research, University of Vienna).

DOC, nutrients and community metabolism — Streamwater N-NO_3 and P-PO_4 concentrations were measured on a continuous flow analyzer (FlowSys 3rd generation, SYSTEA Analytical Technologies) on sterile filtered (0.2µm) samples. DOC concentrations were measured on filtered (pre-combusted Whatmann GFF) samples using a TOC analyzer (Sievers 5310C, GE Analytical Instruments). We acid-washed and combusted (450°C, 4 h) all glassware for DOC analysis prior to sampling. DOC isotopic composition was determined with a LC-IRMS system (HPLC pump connected via LC-Isolink to Delta V Advantage Mass Spectrometer, Thermo Scientific, Department of Terrestrial Ecosystem Research, University of Vienna).

We measured dissolved oxygen (DO) concentration in the microcosms using planar optodes (PSt3 sensor, Presens, Germany). Changes in DO concentration over time (2 – 4 h) during each day-time and night-time incubation were used to determine community metabolic activity.

We used the net-change in O_2 concentration during the light incubations to infer net primary production (NPP) and the net change in O_2 concentration during the dark incubations to infer community respiration (R), assuming a respiration quotient of 0.81 to convert µg O_2 to C

(Berggren et al. 2011, Bengtsson et al. 2014). Respiration during the day was assumed to be equal to the measured respiration at night. We determined GPP as sum of NPP and R (Bott 1996).

Analysis of optical properties of DOC — Absorbance scans and excitation emission matrices (EEMs) of streamwater DOC were generated with an Aqualog® Benchtop Fluorometer (Horriba Scientific); fluorescence intensities were measured at excitation wavelengths ranging from 240 to 450 nm (1-nm increments) and emission wavelengths from 280 to 530 nm (2-nm increments). The water Raman peak of MilliQ water served as reference. We corrected EEMs for blanks (MilliQ) and absorbance (inner filter effect). We modeled individual fluorescent components from the obtained EEMs using parallel factor analysis (PARAFAC) (Stedmon and Bro 2008) using the DOMFluor Toolbox (1.7; containing the N-Way toolbox, 3.1) (Andersson and Bro 2000). PARAFAC identified one humic-like component and two protein-like components (SI Figure 1). Component 1 (Em 439 nm / Ex 321 nm) is indicative of humic material typically of terrestrial origin (Stedmon et al. 2003). Component 2 (Em 339 nm / Ex 300 nm) and component 3 (Em 302 nm / Ex 271 nm) are indicative of tryptophan-like and tyrosine-like fluorescence, and are thought to result from autochthonous production (Stedmon and Markager 2005, Stedmon et al. 2007).

We derived the following suite of optical descriptors from the absorbance and fluorescence measurements: the humification index (HIX) indicative of humic content or of the humification degree of DOC (Zsolnay et al. 1999); the freshness index (ß/α) informs of the freshness of DOC and its potential origin from microbial autochthonous production (Parlanti et al. 2000, Wilson and Xenopoulos 2009); the fluorescence index (FI) reveals sources of DOC (autochthonous

versus allochthonous)(McKnight et al. 2001); the slope ratio (S_R) and the ratio of absorbance at 254 and 365 nm (a_{254}/a_{365}) are inversely correlated with apparent DOC molecular weight (De Haan 1993, Helms et al. 2008); the specific UV absorption ($SUVA_{254}$) is correlated with DOC aromaticity (Weishaar et al. 2003).

Mass balance and C fluxes — We calculated a C mass balance for each microcosm for day-time and night-time incubations based on the difference between the output and input C fluxes for each microcosm. Output fluxes included the DOC concentration at 6 h ($DOC_{t6h,}$ μg C L^{-1}) of the incubations, allochthonous DOC uptake into the biofilm ($U_{alloch,}$ μg C cm^{-2} h^{-1}) and community respiration (R), which we partitioned into respiration of allochthonous ($R_{alloch,}$ μg C cm^{-2} h^{-1}) and autochthonous ($R_{auto,}$ μg C cm^{-2} h^{-1}) C sources. Input C sources for the mass balance included the DOC streamwater concentration at 0 h (DOC_{t0h}, μg C L^{-1}) and exudation (E, μg C cm^{-2} h^{-1}) of DOC from the biofilms. Using these criteria, we calculated the mass C budget ($C_{budget,}$ μg C cm^{-2} h^{-1}) such as

$$C_{budget} = \left(DOC_{t6h} \times \frac{V}{A \times T} + U_{alloch} + R_{alloch} + R_{auto}\right) - \left(DOC_{t0h} \times \frac{V}{A \times T} + E\right) \quad \text{Eq. 1}$$

where $V/(A \times T)$ converts DOC concentration (μg C L^{-1}) to units of μg C cm^{-2} h^{-1} as V is the volume of the chambers (0.75 L), A is the total surface area (cm^2) of all slides present in the microcosm at the given time and T is time (h).

To partition the autochthonous and allochthonous C sources to calculate the mass balance for each of our microcosms (Eq. 1), we used the concentration-dependent isotope-mixing model "Stable Isotope Analysis in R" (SIAR) (Parnell et al. 2008). The $\delta^{13}C$ from the willow extract in the streamwater and the biofilm biomass served as end-members for SIAR. We assumed a

fractionation factor of 1 as microbial heterotrophs typically have the same $\delta^{13}C$ signature as the substrate they degrade (Coffin et al. 1989). Detailed information on the SIAR parameters are given in the Supplementary Information (SI Tables 2-4). We inferred the relative contributions from allochthonous and autochthonous sources to the DOC pool for three day-time and night-time incubations.

First, we calculated U_{alloch} (μg C cm^{-2} h^{-1}) based on the difference in DOC concentration over the incubation time and the proportion of allochthonous DOC such as

$$U_{alloch} = DOC_{t0h} - (pDOC_{alloch} \times DOC_{t6h}) \times \frac{V}{(A \times T)}$$

Eq. 2

where $pDOC_{alloch}$ is the relative contributions (%) of allochthonous DOC to the streamwater DOC pool as estimated from the SIAR mixing model. To infer the allochthonous and autochthonous sources contributing to total community respiration (R, μg C cm^{-2} h^{-1}), we assumed R_{alloch} (μg C cm^{-2} h^{-1}) was the remainder of the total uptake of streamwater DOC after subtracting the amount of C assimilated into the biofilm where

$$R_{alloch} = U_{alloch} - BGE \times U_{alloch}$$

Eq. 3

and BGE is the bacterial growth efficiency for streams (25%, del Giorgio and Cole 1998). To calculate the contribution of community respiration from authocthonous sources of C (R_{auto}, μg C cm^{-2} h^{-1}), we assumed that it would be the difference between community R and R_{alloch} where

$$R_{auto} = R - R_{alloch}$$

Eq. 4

And finally to calculate the input fluxes besides the streamwater input, we calculated the exudation flux (E, μg C cm^{-2} h^{-1}) from the biofilms based on the proportion of autochthonous DOC of the final DOC concentration at the end of the incubations where

$$E = pDOC_{auto} \times DOC_{t6h} \times \frac{V}{A \times T} \quad \text{Eq. 5}$$

where $pDOC_{auto}$ (%) is the proportion of autochthonous DOC of the total DOC concentration at hour 6 of the incubations as calculated using the SIAR model.

Extracellular enzyme activities — We measured the activity of leucine-aminopeptidase (EC 3.4.11.1), phosphatase (EC 3.1.3) and beta-glucosidase (EC 3.2.1.21) using aminomethylcoumarin (AMC) and methylumbelliferyl (MUF) (Sigma Chemical Company) as detailed in Wagner et al. (2015). We also measured phenol oxidase activity (EC 1.14.18.1) using 3,4-dihydroxyphenylalanine (L-DOPA). These measures were done on biofilms from three day-time (corresponding to time points 32, 80 and 128 h) and night-time incubations (corresponding to time points 44, 92 and 140 h).

Data analyses — All data analyses were performed using R (R Development Core Team, 2015). The concentration-dependent isotope-mixing model was computed using the 'siar' package (Parnell et al. 2008). The relationship between NPP and light intensity was described with a hyperbolic tangent function ($NPP = P_{max} \times \tanh(\alpha \times I / P_{max})$, where P_{max} is light saturated photosynthesis, α is the slope of the initial P-I curve and I is the light intensity) by non-linear regression (Jassby and Platt 1976). Biomass, chlorophyll *a* and extracellular enzyme activities depicted similar relationships with light intensity, and we therefore used the same saturation

247 model on these relationships. We fitted non-linear regressions using the nls function. Principal
248 component analysis (PCA) on $SUVA_{254}$, HIX, FI, S_R, ß/α, and a_{254}/a_{365} and the PARAFAC
249 components one, two and three was used to explore possible changes of DOC composition
250 during incubations. We focused on the fluorescence and absorbance measures of three day-time
251 (corresponding to time points of 32, 56 and 80 h) and night-time incubations (corresponding to
252 time points of 44, 68 and 92 h) for the PCA to assure overlap with results from the SIAR model.
253 We explored the effect of measured light intensities, beta-glucosidase and phenol oxidase
254 activities on respiration and uptake of allochthonous DOC using general additive models (GAM)
255 with a residual temporal correlation structure (auto-regressive model of order 1). Spearman's
256 rank correlation between pairs of predictor variables had correlation coefficients lower than ±0.5
257 and the calculated variance inflation factors were lower than 1.5 (Booth et al. 1994), indicating
258 non-collinearity among predictor variables. We fitted GAMs using the "mgcv" package (Wood
259 2011).
260
261 **Results.**
262 *Biomass, NPP and R* — Chlorophyll *a*, cell abundance and biofilm biomass showed saturating
263 relationships with increasing light intensity; this indicates that we were able to establish an
264 increasing gradient of algal biomass (Table 1). A further increase in light intensity would not
265 have likely stimulated further biomass accrual as the highest light treatment (92%T) already
266 instigated a biomass decrease (SI Figure 2), indicative of photo-inhibition or nutrient limitation.
267 NPP and R fluxes significantly increased during the 140 h of the experiment (ANOVA, NPP: P
268 < 0.001, $F_{5,174} = 14.06$; R: $P < 0.001$, $F_{5,171} = 19.4$) in all light treatments (Figure 1) and
269 significantly differed across light treatments (ANOVA, NPP: $P < 0.001$, $F_{5,174} = 35.3$; R: $P <$

0.001, $F_{5,171}$ = 30.9). A net release of DOC was observed in all light treatments during day-time and night-time incubations and ranged from 0.01 to 1 µg C cm^{-2} h^{-1}. Streamwater N-NO_3 concentration averaged 13.0±3.6 µg cm^{-2} and average P-PO_4 concentration was 0.2±0.2 µg cm^{-2} at the end (t_{6h}) of the incubations and significantly differed across the light treatments (ANOVA, PO_4: $P < 0.001$, $F_{5,354}$ = 181.6; NO_3: $P < 0.001$, $F_{5,354}$ = 21.5). Streamwater N-NO_3 concentration was not fully depleted in any of the light treatments, whereas P-PO_4 concentration was completely removed from the streamwater during the incubations in the treatments with high light availability (69%T and 92%T) (SI Table 5).

Streamwater DOC composition — The results of the PCA indicated that DOC composition as inferred from optical properties did not differ among light treatments for neither day-time nor night-time incubations (Figure 2). However, we found that DOC composition changed during day-time incubations (ANOVA, $P < 0.001$, $F_{6,90}$ = 6.1; separation of streamwater and light treatments on the first PCA axis). During night-time incubations the first PCA axis suggests a separation of DOC composition from the streamwater and the 69%T, the 51%T and the 24%T light treatments (ANOVA, $P < 0.001$, $F_{6,93}$ = 5.5).

C budget and fluxes in benthic biofilms — Light availability significantly influenced the microcosm C budget (C_{budget}) and DOC fluxes (Table 2). The C budgets were significantly lower in night-time incubations across all light treatments ($P < 0.05$, t = 2.2, df = 150); benthic biofilms produced DOC in all light treatments during day-time and night-time incubations (Figure 3, SI Figure 3).

Light and NPP drove allochthonous C uptake (U_{alloch}) and R. Allochthonous C uptake decreased across the light gradient during day-time and more so during night-time incubations with highest uptake of allochthonous DOC under low light conditions (Figure 4). Furthermore, allochthonous DOC uptake decreased with increasing NPP (Figure 5b). This decrease in allochthonous DOC uptake could explain the increase in R during the day (Figure 5c), which may be indicative of the organic carbon supply from autochthonous sources rather than allochthonous sources in treatments with high light intensities.

The contribution of autochthonous DOC to biofilm respiration increased significantly with light intensity (day: $r^2 = 0.60$, $P < 0.001$, df = 88; night: $r^2 = 0.68$, $P < 0.001$, df = 85) (SI Figure 4a, b); it averaged from 34% to 93% in treatments with low and high light intensities (7%T and 92%T), respectively (Table 3). Conversely, allochthonous DOC contributed on average 7% to 66% to respiration in treatments with high and low light intensity (92%T and 7%T), respectively (Table 3) and its contribution significantly decreased with enhanced light availability (day: $R^2 = 0.20$, $P < 0.001$, df = 88; night: $R^2 = 0.14$, $P < 0.01$, df = 85) (SI Figure 4c, d).

As for exudation fluxes, the results of non-linear regression analyses indicated statistically significant saturating relationships between exudation rates (E) and light intensity for some of the incubations, although the r^2 was relatively low ($r^2 < 0.1$) for most days (Figure 6). Increased NPP could explain increased exudation (E) rates across the light gradient as there was a significant saturating relationship, although the $r^2 = 0.04$ indicates a weak relationship of exudation and production within the biofilms (Figure 5a).

Extracellular enzymatic activity and DOC dynamics — Phenol oxidase activity decreased along the light gradient in both day-time and night-time incubations (Figure 7a-b). Conversely, beta-

glucosidase, leucine-aminopeptidase and phosphatase activities showed significantly saturating relationships with light intensity (Figure 7c-h). We also found that enzyme activities significantly increased during the duration of the experiment (ANOVA, $P < 0.001$, df = 5) and phenol oxidase (ANOVA, $P < 0.001$, $F_{1,158} = 61.2$) and leucine-aminopeptidase activities (ANOVA, $P < 0.05$, $F_{1,177} = 4.3$) were significantly higher at night than during the day. Furthermore, glucosidase activity was significantly related to respiration (GAM, deviance explained = 56.1%, $P < 0.01$, $n = 75$) and phenol oxidase activity was significantly related to the uptake of allochthonous DOC (GAM, deviance explained = 36.3%, $P < 0.001$, $n = 78$) (Table 4).

Discussion.

The study of light as a major driver of benthic algal community composition and metabolism has been a mainstay in stream ecology (Hill 1996). Combining stable isotope analyses with end-member mixing models and extracellular enzymatic activities, our findings show how light intensity modulates exudation and the potential contributions of allochthonous and autochthonous carbon to community respiration of benthic biofilms; they did not support priming as relevant in these communities. These fluxes are potentially critical for C cycling in stream ecosystems.

Phototrophic biofilms and DOC composition — Our estimates of GPP fluxes (high light (92%T): 1.6±0.6 g C m^{-2} d^{-1}; low light (7%T): 0.4±0.2 g C m^{-2} d^{-1}) are closely bracketed by measurements from stream ecosystems (e.g. Wiley et al. 1990, Hall 1972, Bott et al. 1985) and similar to GPP values from streams with seasonal variability in PAR intensity (Acuña et al. 2004, Young and Huryn 1999) and differing riparian canopy cover (Mulholland et al. 2001). Community

respiration (R) rates of 0.1±0.04 to 1.4±0.1 g C m^{-2} d^{-1} in the low and high light treatments (7%T and 92%T) fall within the lower range of field-based measurements in streams (e.g. Hall 1972, Bott et al. 1985, Uehlinger and Naegeli 1998, Young and Huryn 1999). Average GPP to R ratios of 3.5±0.6 and 4.3±0.5 in low and high light treatments (7%T and 92%T), respectively underscore the autotrophic character of the benthic biofilms used in our study. Our findings suggest that these autotrophic biofilms imparted the streamwater DOC composition through release of algal exudates to the streamwater. In fact, streamwater DOC was initially (that is at t_{0h}) characterized by an elevated degree of humification as indicated by increased HIX values. Notably, DOC in the light treatments (that is at t_{6h}) had increased contributions from the amino acid-like fluorescent components C2 and C3, and possibly in situ production of fresh, low-molecular weight compounds (as suggested by β/α and S_R). This agrees with ecosystem-level observations from the same stream (Oberer Seebach) showing increased autochthonous signatures in streamwater DOC during extended baseflow and elevated primary production (Fasching et al. 2015). DOC aromaticity (from $SUVA_{254}$) increased during the incubations, which is likely attributable to the transformation of algal exudates by bacteria. This is consistent with the perception of the dual role of bacteria consuming labile DOC compounds and at the same time producing refractory DOC compounds (Romera-Castillo et al. 2011, Guillemette and del Giorgio 2012, Jiao et al. 2010).

Light intensity modulates the uptake dynamics of allochthonous versus autochthonous DOC — Understanding the relative consumption of allochthonous versus autochthonous DOC by microbial heterotrophs is important as it may influence overall carbon fluxes in stream ecosystems. It has been common wisdom that allochthonous DOC has low turnover and

361 therefore constitutes a constant reservoir to microbial heterotrophs in freshwater ecosystems
362 (e.g., Wetzel 2002). Studies that combine flux measurements with radiocarbon dating of respired
363 carbon confirm this notion and highlight the fact that even ancient allochthonous DOC is
364 degraded by microbial heterotrophs (e.g., McCallister and del Giorgio 2008). On the other hand,
365 autochthonous and labile DOC constitutes a more stochastic energy source to microbial
366 heterotrophs and apparently conveys less stability to ecosystem metabolism than allochthonous
367 DOC (e.g., Wetzel 2002). Our findings suggest that light intensity modulates the relative uptake
368 of allochthonous versus autochthonous DOC and its contribution to respiration by benthic
369 biofilms. Under low light availability (7%T) benthic biofilms degraded allochthonous DOC as
370 the major energy source, whereas autochthonous DOC was preferentially degraded under high
371 light availability (92%T, 69%T, 51%T). Interestingly, the mass balance results showed that all
372 light treatments were net sources of DOC owing to elevated exudation rates and partial leaking
373 of algal exudates into the streamwater. However the relationship between light intensity and
374 exudation was weak given the low r^2, which could potentially be explained by increased internal
375 C cycling within benthic biofilms under high light availability as freshly produced C from
376 primary producers is fast taken up by the microbial heterotrophs (Romaní et al. 2004, Ylla et al.
377 2009). This fast C cycling within the benthic biofilms may partially correct for the missing C
378 flux of autochthonous DOC uptake for the mass balance calculations.
379 Furthermore, nutrient availability has been reported as a control on the uptake dynamics of
380 allochthonous and autochthonous DOC by stream biofilms (Ziegler et al. 2009). These authors
381 found that biofilms growing in nutrient-rich streamwater released more DOC from algal
382 leachates than biofilms growing in nutrient-poor streamwater. We adjusted initial nutrient
383 concentrations to equality across experimental incubations to test for effects of light intensity on

384 the carbon fluxes in benthic biofilms. Thereby, we were able to detect signatures of phosphate
385 limitation in those biofilms exposed to treatments with high light intensity (69%T and 92%T).
386 This could potentially explain the observed decrease in community NPP, R and extracellular
387 enzymatic activity under elevated light availability. It is reasonable to assume that
388 microorganisms experiencing phosphorus limitation up-regulated the production of phosphatase
389 to further mobilize inorganic phosphorus (Jansson et al. 1988) from the autochthonous DOC
390 pool.
391
392 *No priming of allochthonous DOC by autochthonous DOC* — Algal and bacterial cells co-exist
393 in close spatial proximity in benthic biofilms, with algal exudates potentially stimulating
394 interactions between these organisms (e.g., Romaní and Sabater 1999, Rier and Stevenson 2002,
395 Rier et al. 2014). This configuration offers potential for priming of allochthonous DOC by labile
396 organic compounds from algal exudates as reported from a handful of studies on aquatic DOC
397 degradation in benthic systems (e.g., Farjalla et al. 2009, vanNugteren et al. 2009). Our findings
398 did not support the occurrence of priming in stream benthic biofilms. Rather they show that the
399 uptake of allochthonous DOC decreased with increasing light intensity and even diminished as
400 NPP increased. This pattern is likely explained by preferential uptake of autochthonous DOC
401 over allochthonous DOC by microbial heterotrophs. This notion is supported by the observation
402 that the expression of leucine-aminopeptidase and beta-glucosidase was putatively stimulated in
403 biofilms with elevated algal biomass and exudation rates. This is consistent with earlier reports
404 on the degradation of autochthonous DOC in benthic stream biofilms (Haack and McFeters
405 1982, Romaní et al. 2004, Rier et al. 2014). Furthermore, biofilm respiration was fueled to a
406 large extend by autochthonous DOC during the day when light intensity was high; this reversed

407 at night when the allochthonous DOC apparently contributed more to respiration. Interestingly,
408 beta-glucosidase activity was expressed at similar rates in treatments with elevated light intensity
409 (51%T, 69%T and 92T%) at day and during the night. This may be attributable to beta-
410 glucosidase breaking down autochthonous DOC at day and possibly being involved in the later
411 degradation steps of allochthonous DOC during the night (Deshpande and Eriksson 1988). A
412 shift from autochthonous DOC to predominantly allochthonous DOC was observed during the
413 night in all light treatments. This suggests enhanced internal cycling of organic carbon from algal
414 exudates and their lysis products at day and possibly reduced phototrophic-heterotrophic
415 interactions during the night (Romaní and Sabater 1999, Ylla et al. 2009). At the same time the
416 activity of phenol oxidase, associated with the degradation of refractory allochthonous DOC
417 (Sinsabaugh 2010), was higher at night than during the day and induced an elevated night-time
418 uptake of allochthonous DOC.
419 Our observations on the absence of priming in benthic biofilms are consistent with other studies
420 that were not able to unequivocally demonstrate priming as a major process in freshwater
421 microbial communities (e.g., Bengtsson et al. 2014, Catalán et al. 2015). Priming seems to be
422 relevant though in aquatic ecosystems dominated by leaf litter breakdown (Kuehn et al. 2014),
423 which are more similar to soil systems than to phototrophic biofilms because of their large pool
424 of particulate organic matter. In fact, priming has been originally reported from soils where it
425 apparently plays a major role in carbon and nutrient cycling (Blagodatskaya and Kuzyakov 2008,
426 Kuzyakov 2010). Our findings do however confirm work on lake microbial communities
427 showing the preferential uptake of algal-derived DOC under certain conditions (Kritzberg et al.
428 2004, Guillemette et al. 2013).

Ecosystem implications — Our findings underscore light as an important modulator of carbon fluxes in benthic biofilms providing mechanistic insights that have implications for stream ecosystems. Algae in biofilms likely exudate less DOC under low light availability and microbial heterotrophs largely satisfy their carbon demand from allochthonous sources, whereas internal carbon cycling over small time scales as initiated by algal exudates gains relevance as more light is available. Light regime fluctuates seasonally and diurnally in streams and more important also longitudinally from the smallest headwaters to larger downstream reaches, and depending on shading also within a given reach. This translates into a temporal and spatial distribution of light with hitherto unresolved consequences for carbon fluxes. Our experimental findings in fact suggest different metabolic fates for allochthonous and autochthonous DOC in streams depending on light intensity. This may have impacts on streamwater DOC quality and its delivery to downstream ecosystems.

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Tables.

Table1. Mean ± standard deviation of microbial biomass in benthic biofilms across light treatments (transmission of the incident light (%T)) after 8 and 128 hours of the experiment.

Time	Parameter	7%T	14%T	24%T	51%T	69%T	92%T
8h	Chlorophyll *a* (µg cm^{-2})	0.9±0.5	2.8±0.9	3.1±0.8	2.4±0.8	2.1±0.9	2.1±1.0
	Biomass (µg C cm^{-2})	110±40	115±110	290±200	230±120	600±550	370±320
	Cell abundance (10^7 cm^{-2})	1.8±0.5	2.7±1.1	3.8±1.4	4.1±1.9	3.8±1.5	3.1±1.9
128h	Chlorophyll *a* (µg cm^{-2})	2.0±0.5	3.2±1.1	4.0±0.4	3.7±0.4	3.7±0.7	3.8±0.7
	Biomass (µg C cm^{-2})	60±30	130±60	310±220	210±30	420±120	370±130
	Cell abundance (10^7 cm^{-2})	3.4±1.1	6.0±0.9	7.9±2.1	8.0±2.7	10.1±2.6	9.0±1.2

666 Table2. Mean ± 95% confidence interval (minimum to maximum) for the mass fluxes and C
667 budget (µg C cm^{-2} h^{-1}) in the microcosms for three day (32, 56 and 80 h) and night incubations
668 (20, 44 and 68 h) across all light treatments (transmission of the incident light (%T)).

Parameter	**7%T**	**14%T**	**24%T**	**51%T**	**69%T**	**92%T**
NPP (day)	1.1±0.3 (0.5 to 2.4)	1.9±0.3 (0.9 to 3.0)	3.0±0.3 (2.3 to 4.0)	3.7±0.6 (1.8 to 5.4)	4.0±0.5 (2.8 to 5.7)	4.3±0.7 (2.1 to 5.9)
NPP (night)	NA	NA	NA	NA	NA	NA
E (day)	0.6±0.1 (0.5 to 1.0)	0.8±0.1 (0.5 to 1.3)	0.7±0.1 (0.6 to 1.5)	0.8±0.1 (0.6 to 1.1)	0.9±0.1 (0.6 to 1.4)	0.6±0.1 (0.4 to 0.8)
E (night)	0.8±0.1 (0.5 to 1.0)	0.9±0.1 (0.7 to 1.2)	0.9±0.1 (0.7 to 1.2)	0.9±0.1 (0.8 to 1.1)	0.8±0.1 (0.6 to 1.1)	0.8±0.1 (0.6 to 1.1)
R (day)	0.4±0.1 (0.2 to 0.7)	0.6±0.1 (0.4 to 0.8)	0.9±0.1 (0.7 to 1.3)	1.1±0.1 (0.9 to 1.7)	1.2±0.2 (0.8 to 2.0)	1.3±0.2 (0.9 to 2.4)
R (night)	0.4±0.1 (0.2 to 0.7)	0.6±0.1 (0.4 to 1.3)	0.9±0.1 (0.6 to 1.3)	1.1±0.1 (0.8 to 1.7)	1.1±0.2 (0.7 to 2.0)	1.2±0.2 (0.8 to 2.4)
U_{alloch} (day)	0.4±0.1 (0.2 to 0.6)	0.4±0.1 (0.1 to 0.6)	0.3±0.1 (0.0 to 0.6)	0.2±0.1 (0.0 to 0.6)	0.2±0.1 (0.0 to 0.6)	0.1±0.1 (0.0 to 0.5)
U_{alloch} (night)	0.6±0.1 (0.4 to 0.8)	0.6±0.1 (0.4 to 0.8)	0.5±0.1 (0.4 to 0.8)	0.5±0.1 (0.3 to 0.7)	0.5±0.1 (0.2 to 0.7)	0.4±0.1 (0.3 to 0.6)
C_{budget} (day)	0.5±0.1 (0.3 to 0.7)	0.6±0.1 (0.4 to 0.8)	0. 9±0.1 (0.7 to 1.3)	1.1±0.1 (0.7 to 1.7)	1.2±0.2 (0.8 to 2.0)	1.3±0.2 (0.9 to 2.4)
C_{budget} (night)	0.6±0.1 (0.4 to 0.8)	0.6±0.1 (0.4 to 1.3)	0.8±0.1 (0.6 to 1.0)	1.0±0.1 (0.8 to 1.5)	1.0±0.1 (0.7 to 1.4)	1.1±0.1 (0.8 to 1.4)

Abbreviations: Net primary production (NPP), exudation (E), community respiration (R), allochthonous DOC uptake (U_{alloch}), C mass balance (C_{budget})

669

Table3. Mean ± 95% confidence intervals (minimum to maximum) of the percentage (%) from autochthonous ($pDOC_{auto}$) and allochthonous ($pDOC_{alloch}$) sources of DOC contributing to the respiration in the microcosms for three day (32, 56 and 80 h) and night incubations (20, 44 and 68 h).

Parameter	**7%T**	**14%T**	**24%T**	**51%T**	**69%T**	**92%T**
$pDOC_{alloch}$ (day)	65.9±15.0 (29.0 to 100)	48.9±14.0 (11.4 to 91.2)	24.0±11.0 (1.6 to 58.4)	16.3±6.9 (0.7 to 45.1)	17.2±8.9 (0.0 to 54.3)	7.2±6.0 (0.0 to 34.6)
$pDOC_{alloch}$ (night)	91.4±8.7 (54.1 to 100)	83.1±10.6 (39.3 to 100)	55.6±8.3 (29.1 to 79.5)	37.6±6.1 (12.3 to 54.0)	42.2±7.7 (23.2 to 70.1)	32.2±3.7 (18.7 to 44.9)
$pDOC_{auto}$ (day)	34.1±15.0 (0 to 71.0)	51.1±14.0 (8.8 to 88.6)	76.0±11.0 (41.6 to 98.4)	83.7±6.9 (54.9 to 99.3)	82.8±8.9 (45.7 to 100)	92.8±6.0 (65.4 to 100)
$pDOC_{auto}$ (night)	8.6±8.7 (0 to 45.9)	16.9±10.6 (0 to 60.7)	44.4±8.3 (20.5 to 70.9)	62.4±6.1 (46.0 to 87.7)	57.8±7.7 (29.9 to 76.8)	67.8±3.7 (55.1 to 81.3)

685 Table4. Statistics from the GAM modeling output of community respiration (R, µg C cm^{-2} h^{-1})
686 and allochthonous DOC uptake fluxes (U_{alloch}, µg C cm^{-2} h^{-1}) versus C-acquiring enzymes (nmol
687 cm^{-2} h^{-1}) and light availability (µmole photons m^{-2} s^{-1}) .

Parameter	**Beta-glucosidase**	**Phenol oxidase**	**Light intensity**	**GAM Model statistics**
R (day)	***P* < 0.01**	P > 0.05	**P < 0.001**	R^2_{adj} = 0.54, *n* = 75 deviance explained = 56.1%
R (night)	***P* < 0.01**	P > 0.05	**P < 0.001**	R^2_{adj} = 0.57, *n* = 75 deviance explained = 58.4 %
U_{alloch} (day)	***P* < 0.01**	**P < 0.01**	**P < 0.001**	R^2_{adj} = 0.46, *n* = 78 deviance explained = 47.6%
U_{alloch} (night)	*P* > 0.05	**P < 0.001**	*P* > 0.05	R^2_{adj} = 0.34, *n* = 78 deviance explained = 36.3%

688

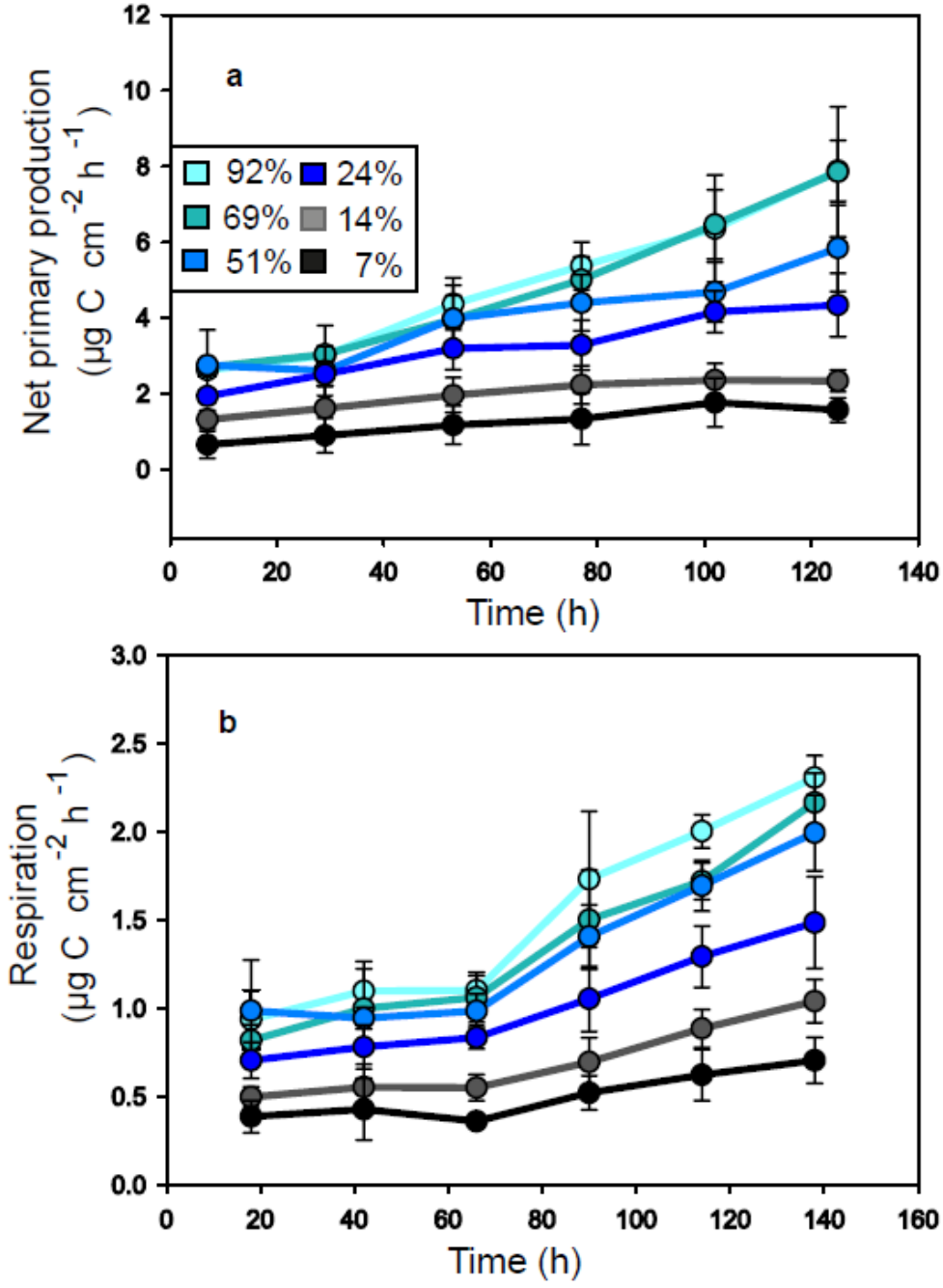


Figure 1. Temporal dynamics of net primary production (NPP, µg C cm^{-2} h^{-1}) (a) and community respiration (R, µg C cm^{-2} h^{-1}) (b) across the light gradient (% transmission (%T) of the incident light).

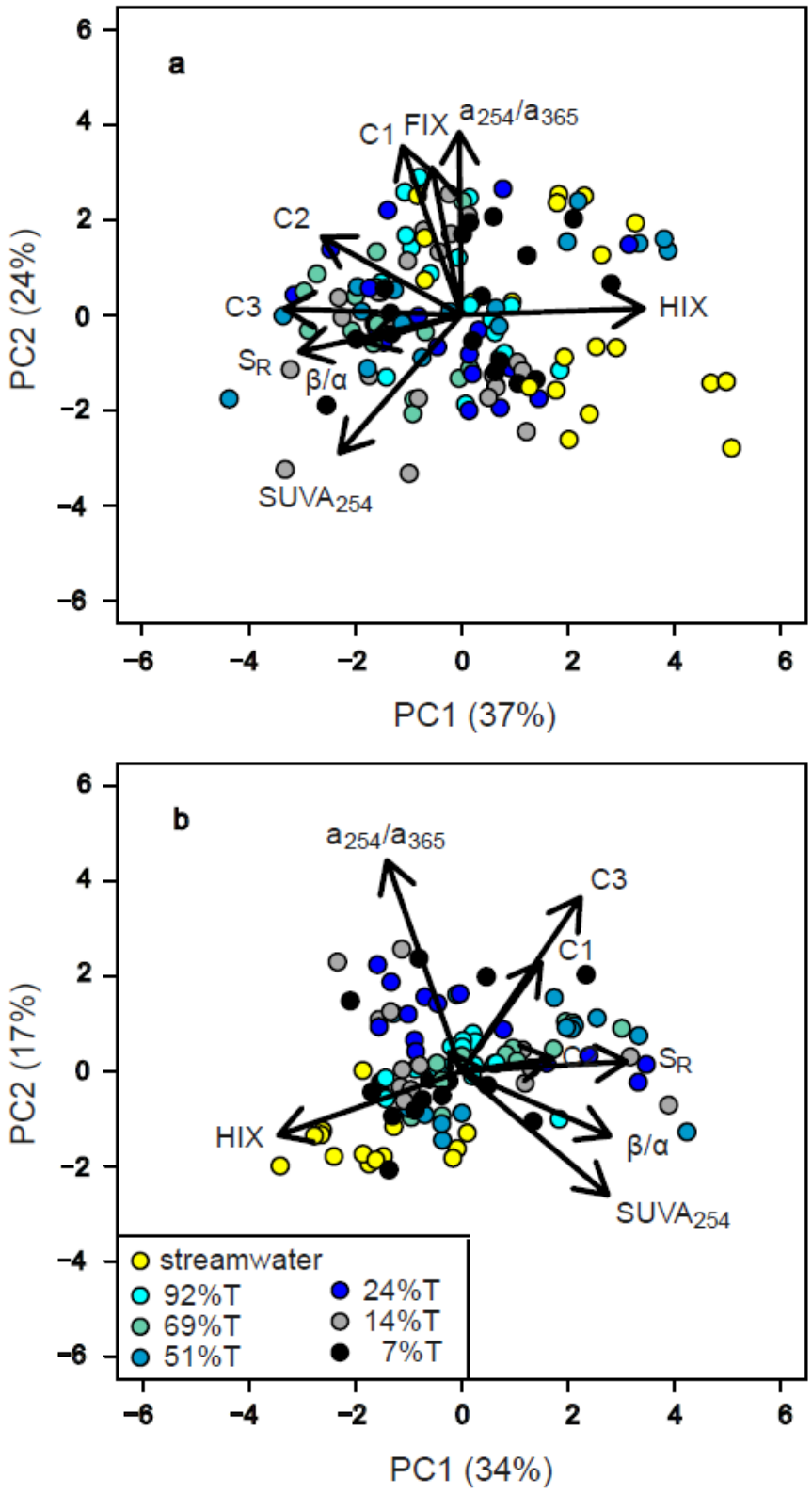


Figure 2. Results of the principal component analysis (PCA) indicate that the streamwater (at t_{0h}) was characterized by a high degree of humification (HIX), whereas elevated aromaticity ($SUVA_{254}$), lower apparent molecular weight compounds (S_R), and the amino acid-like fluorescent components C2 and C3 as well as the freshness index (β/α), were associated with autochthonous processes in the light treatments (% transmission (%T) of the incident light) during day-time (a) and night-time incubations (b); arrows are based on PCA structural coefficients.

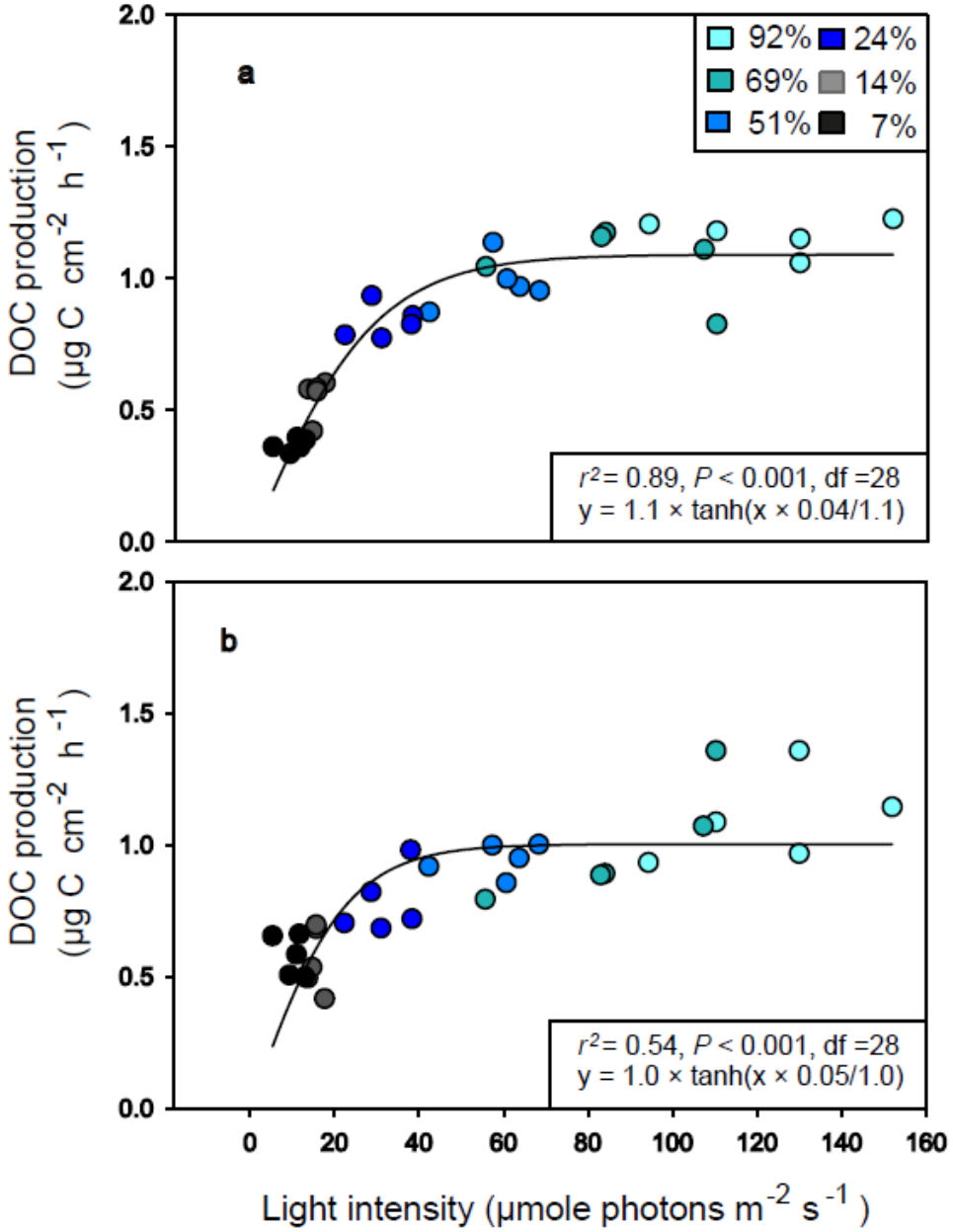


Figure 3. Non-linear regression of the C mass balance (C_{budget}, µg C cm^{-2} h^{-1}) for one representative day (56 h) (a) and night (44 h) incubation (b) along the light gradient (% transmission (%T) of the incident light).

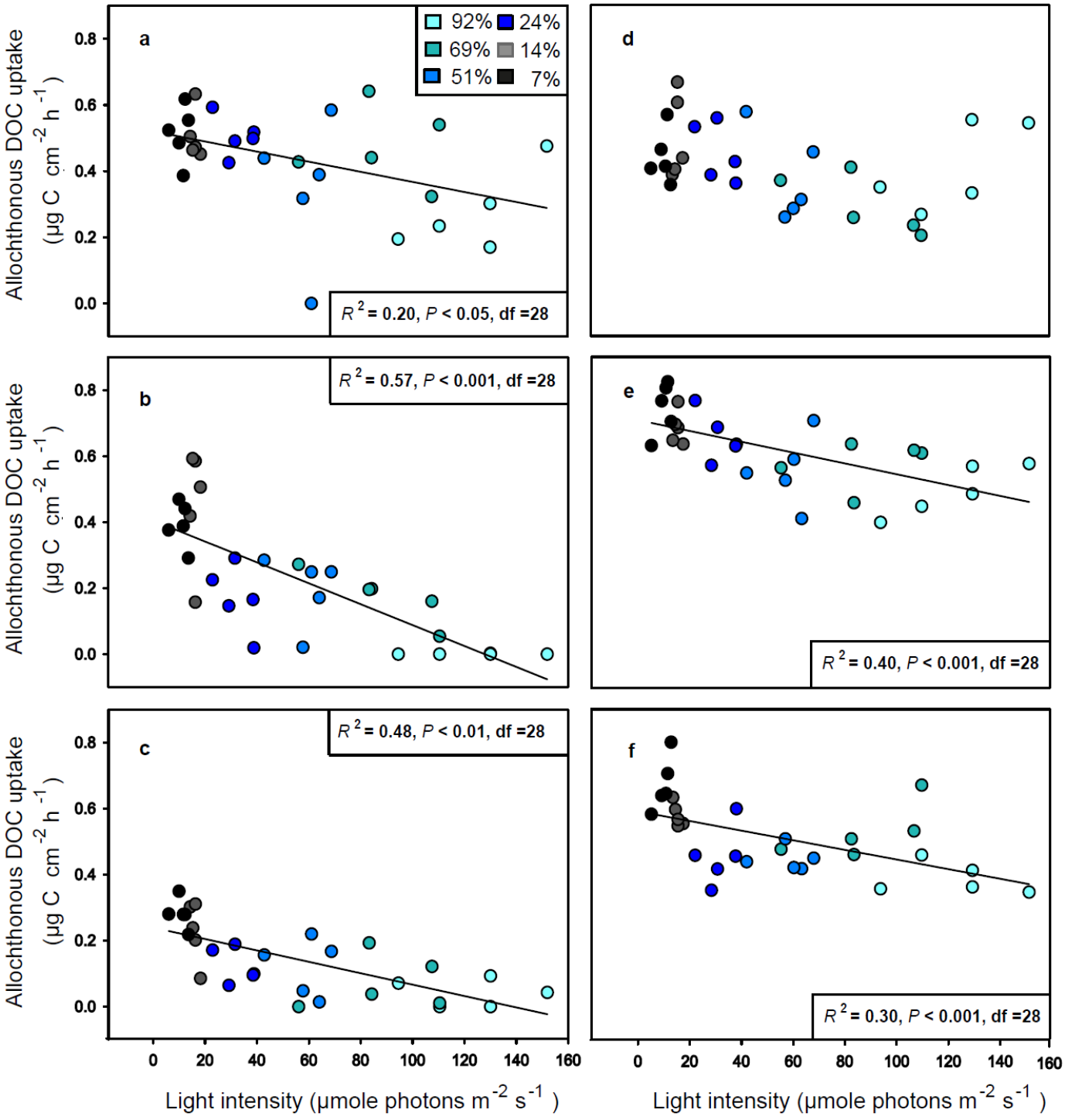


Figure 4. Linear regression of the allochthonous DOC uptake rates across the light gradient (% transmission (%T) of the incident light) during the day (a-c; 32, 56 and 80 h) and the night incubations (d-f; 20, 44 and 68 h).

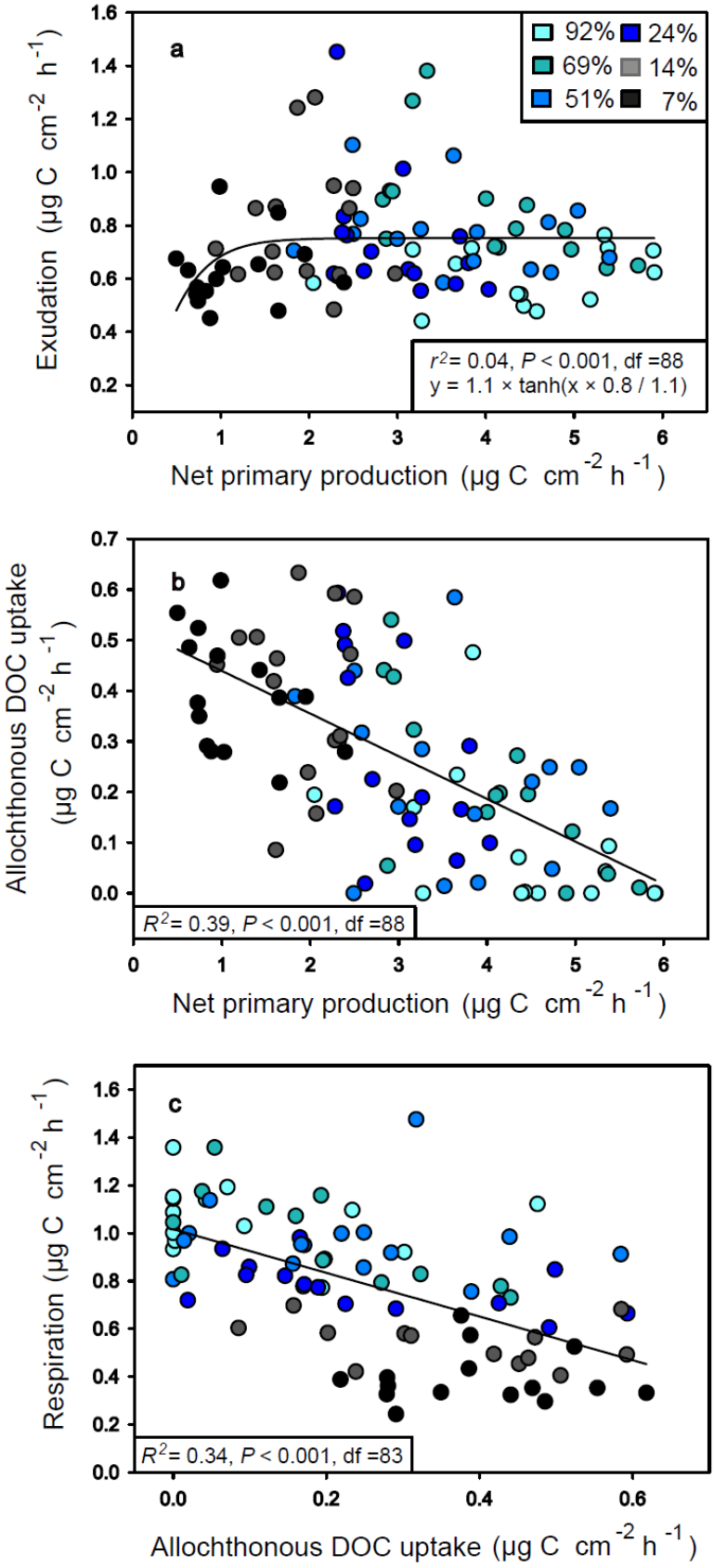


Figure 5. Non-linear regression of modeled exudation fluxes (E, µg C cm^{-2} h^{-1}) and net primary production (NPP, µg C cm^{-2} h^{-1}) (a), linear regression of allochthonous DOC uptake fluxes (U_{alloch}, µg C cm^{-2} h^{-1}) and NPP (b) and linear regression of community respiration fluxes (R, µg C cm^{-2} h^{-1}) and U_{alloch} (c) across the light gradient (% transmission (%T) of the incident light) during the day incubations (32, 56 and 80 h).

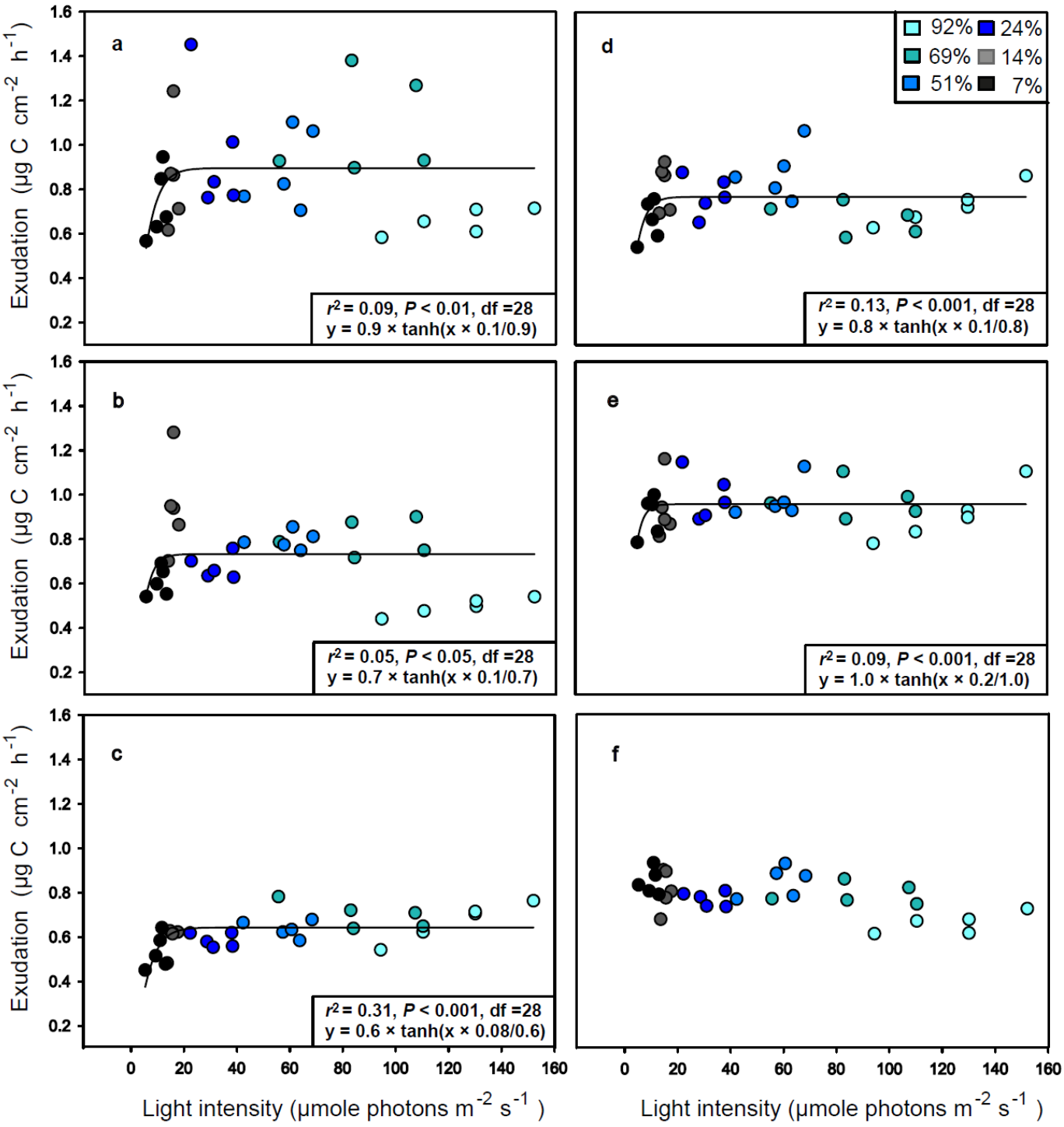


Figure 6. Non-linear regression of exudation rates across the light gradient (% transmission (%T) of the incident light) during the day (a-c; 32, 56 and 80 h) and the night incubations (d-f; 20, 44 and 68 h).

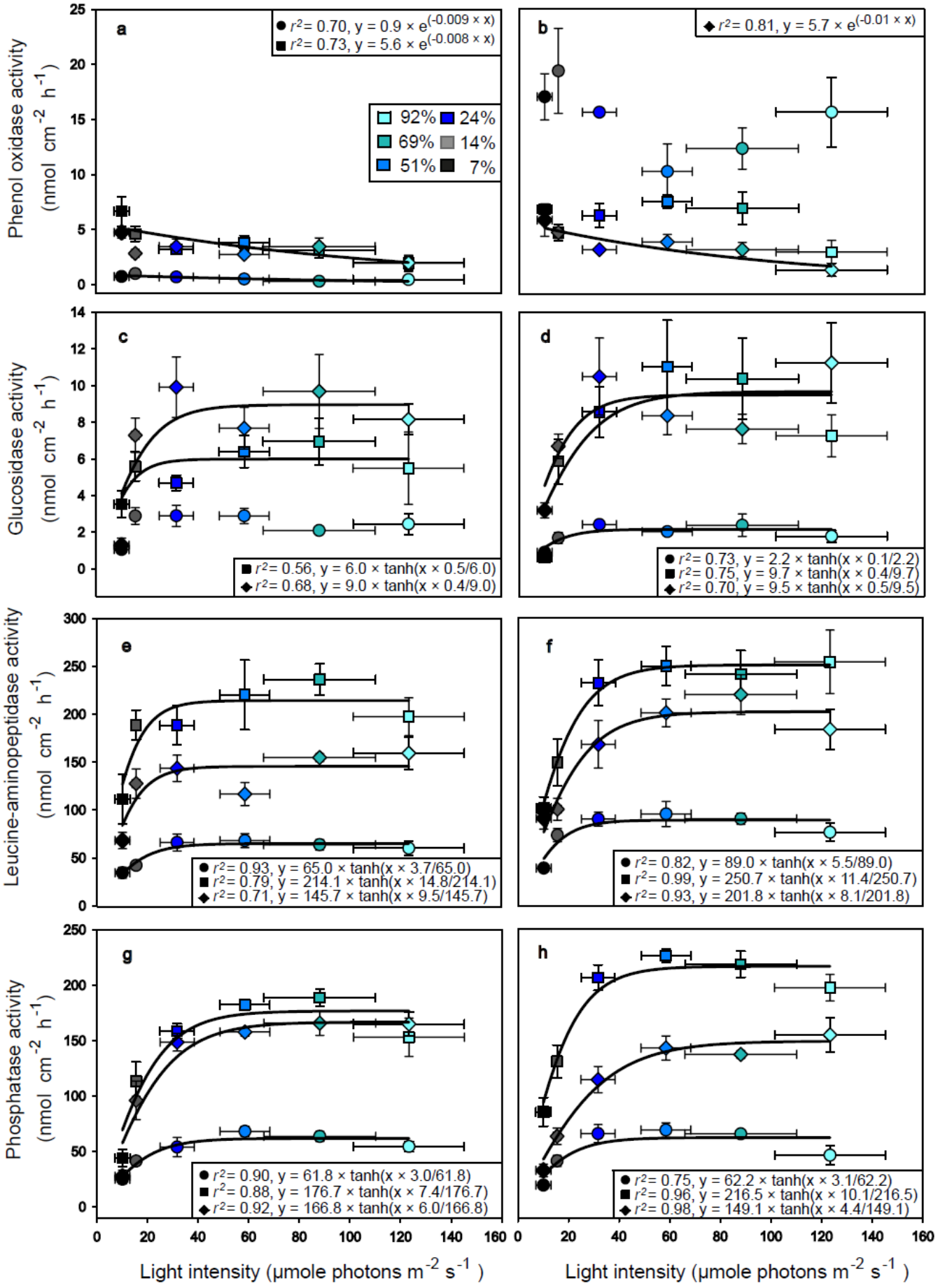


Figure 7. Mean ± standard error and non-linear regression analyses of the activities of phenol oxidase (a-b), beta-glucosidase (c-d), leucine-aminopeptidase (e-f) and phosphatase activities (g-h) during the day (left panel; 32 h (circles), 80 h (squares) and 128 h (diamonds)) and the night incubations (right panel; 44 h (circles), 92 h (squares) and 140 h (diamonds)) across the light gradient (% transmission (%T) of the incident light).

Supplementary Information

SI Table1. Mean ± 95% confidence interval (minimum to maximum) of the nutrient and DOC concentrations at the start (t_{0h}) of the day-time (32, 56, 80, 104 and 128 h) and night-time incubations (20, 44, 68, 92, 116 and 140 h) across all light treatments (transmission of the incident light (%T)).

Parameter	**7%T**	**14%T**	**24%T**	**51%T**	**69%T**	**92%T**
PO_4 t_{0h} (day)	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
(P-PO_4 µg cm^{-2})	(0.5 to 1.1)	(0.5 to 1.1)	(0.5 to 1.1)	(0.5 to 1.1)	(0.5 to 1.1)	(0.5 to 1.1)
PO_4 t_{0h} (night)	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1
(P-PO_4 µg cm^{-2})	(0.6 to 1.2)	(0.6 to 1.2)	(0.6 to 1.2)	(0.6 to 1.2)	(0.6 to 1.2)	(0.6 to 1.2)
NO_3 t_{0h} (day)	14.2±1.2	13.9±1.2	14.2±1.2	14.2±1.2	14.2±1.2	14.2±1.2
(N-NO_3 µg cm^{-2})	(10.4 to 18.4)	(9.7 to 18.4)	(10.4 to 18.8)	(10.3 to 18.7)	(10.4 to 18.4)	(10.3 to 18.4)
NO_3 t_{0h} (night)	15.5 ±1.2	15.1 ±1.2	15.5 ±1.2	15.5 ±1.2	15.5 ±1.2	15.4 ±1.2
(N-NO_3 µg cm^{-2})	(10.8 to 20.5)	(10.1 to 20.5)	(10.9 to 20.9)	(10.7 to 21.0)	(10.8 to 20.5)	(10.7 to 20.5)
DOC t_{0h} (day)	10.5±0.9	10.3±1.0	10.6±0.9	10.5±1.0	10.5±0.9	10.5±0.9
(µg C cm^{-2})	(7.7 to 13.6)	(7.8 to 13.6)	(7.7 to 13.6)	(7.7 to 13.6)	(7.7 to 13.6)	(7.9 to 13.6)
DOC t_{0h} (night)	14.0±0.3	14.2±0.3	12.3±0.5	10.3±0.4	9.1±0.1	8.4±0.3
(µg C cm^{-2})	(12.8 to 15.0)	(13.2 to 15.4)	(10.7 to 13.8)	(9.8 to 12.0)	(8.5 to 9.4)	(7.5 to 9.2)

SI Table2. Parameters used for the SIAR modeling approach; average $DO^{13}C$ signature at the end of the incubations (t_{6h}) across light treatments (% transmission of the incident light (%T)); night-time incubations are indicated by an asterisks.

Time (h)	92%T	69%T	51%T	24%T	14%T	7%T
20 h*	615.3	679.6	591.5	587.6	570.4	625.0
32 h	706.1	555.9	615.4	574.8	591.9	614.0
44 h*	668.2	628.0	630.7	608.5	599.0	599.5
56 h	919.0	788.2	794.7	843.1	711.9	806.1
68 h*	737.2	667.1	677.1	704.4	649.2	604.6
80 h	890.0	881.3	892.2	906.3	885.0	901.1

SI Table3. Parameters used for the SIAR modeling approach; mean±sd of the $\delta^{13}C$ signature from the biofilm biomass and the $DO^{13}C$ signature at the start of the incubations (t_{0h}), which are the two end members in SIAR; night-time incubations are indicated by an asterisks.

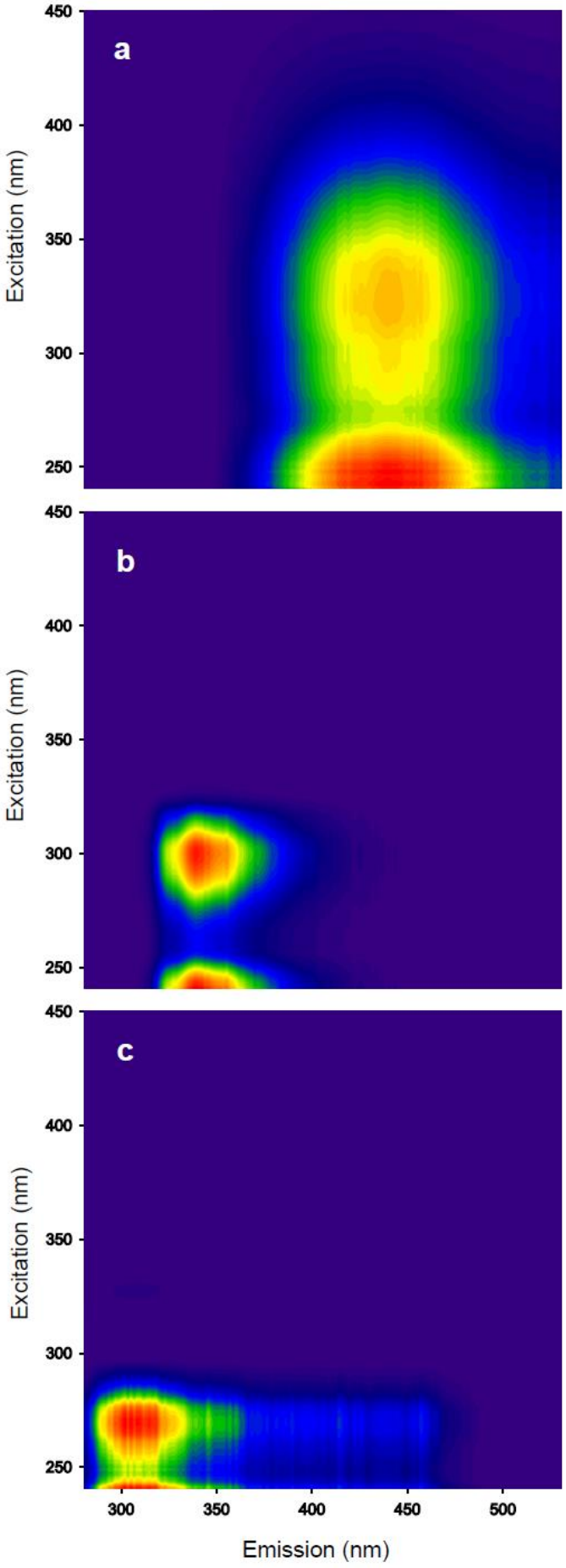
Time (h)	$\delta^{13}C$ Biofilm C	$\delta^{13}C$ DOC
20 h*	160.2±14.4	935.7±150.0
32 h	227.5±16.8	935.7±150.0
44 h*	299.0±22.7	935.7±150.0
56 h	370.4±44.1	935.7±150.0
68 h*	406.7±29.9	935.7±150.0
80 h	443.1±41.6	935.7±150.0

SI Table4. Parameters used for the SIAR modeling approach; mean ± sd of the DOC concentration (g C cm^{-2}) at the start of the incubation (t_{0h}) and biofilm exudation was assumed as 4% of the biofilm biomass (g C cm^{-2}).

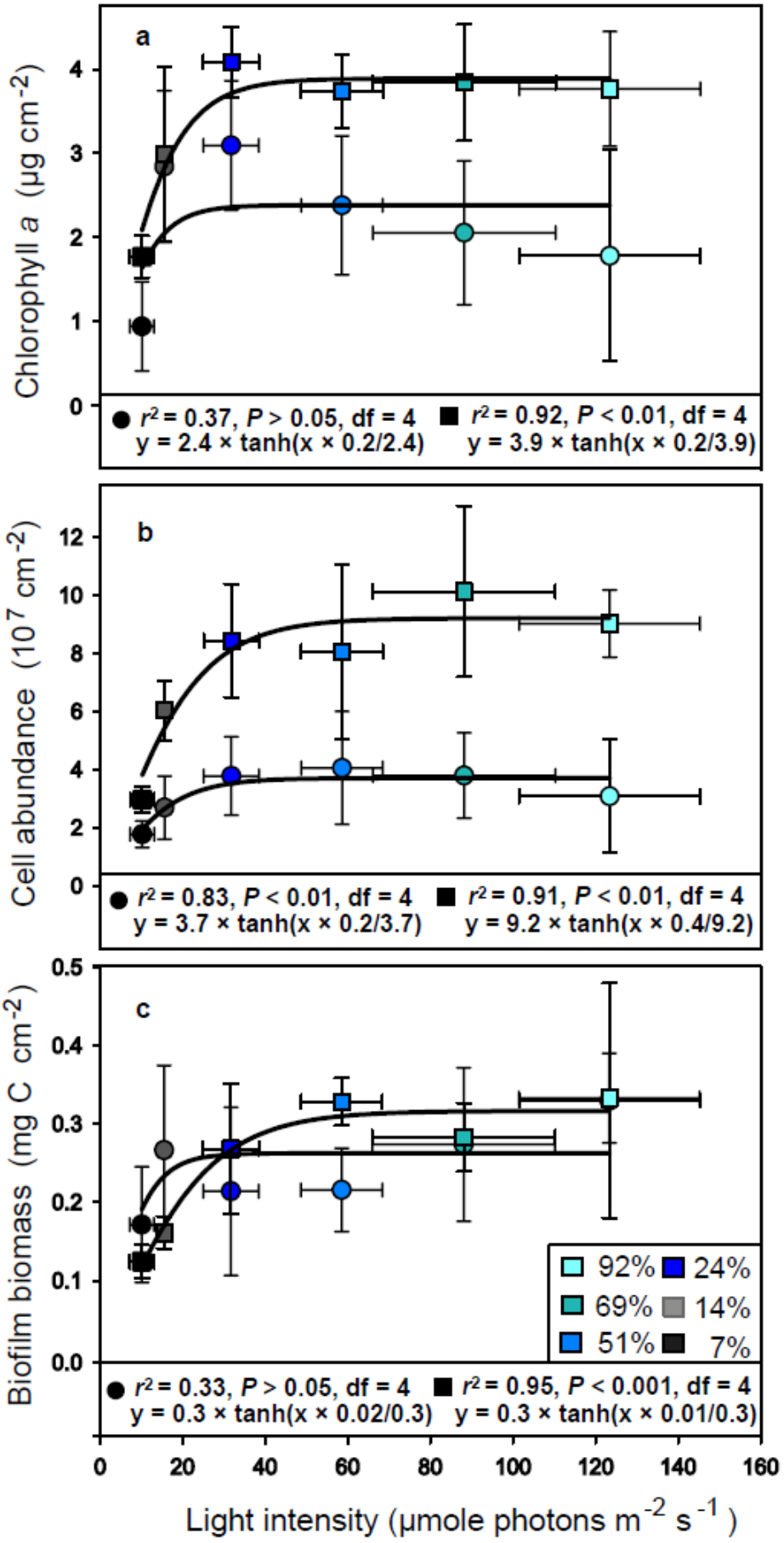
Time	Biofilm exudation	DOC
All time points	0.00059±0.00051	0.00086±0.00010

SI Table5. Mean ± 95% confidence interval (minimum to maximum) of the nutrient concentrations at the end of the incubation (t_{6h}) and nutrient fluxes in the microcosms (0 < indicates nutrient uptake; 0 > indicates nutrient production) for day-time (32, 56, 80, 104 and 128 h) and night-time incubations (20, 44, 68, 92, 116 and 140 h) across all light treatments (transmission of the incident light (%T)).

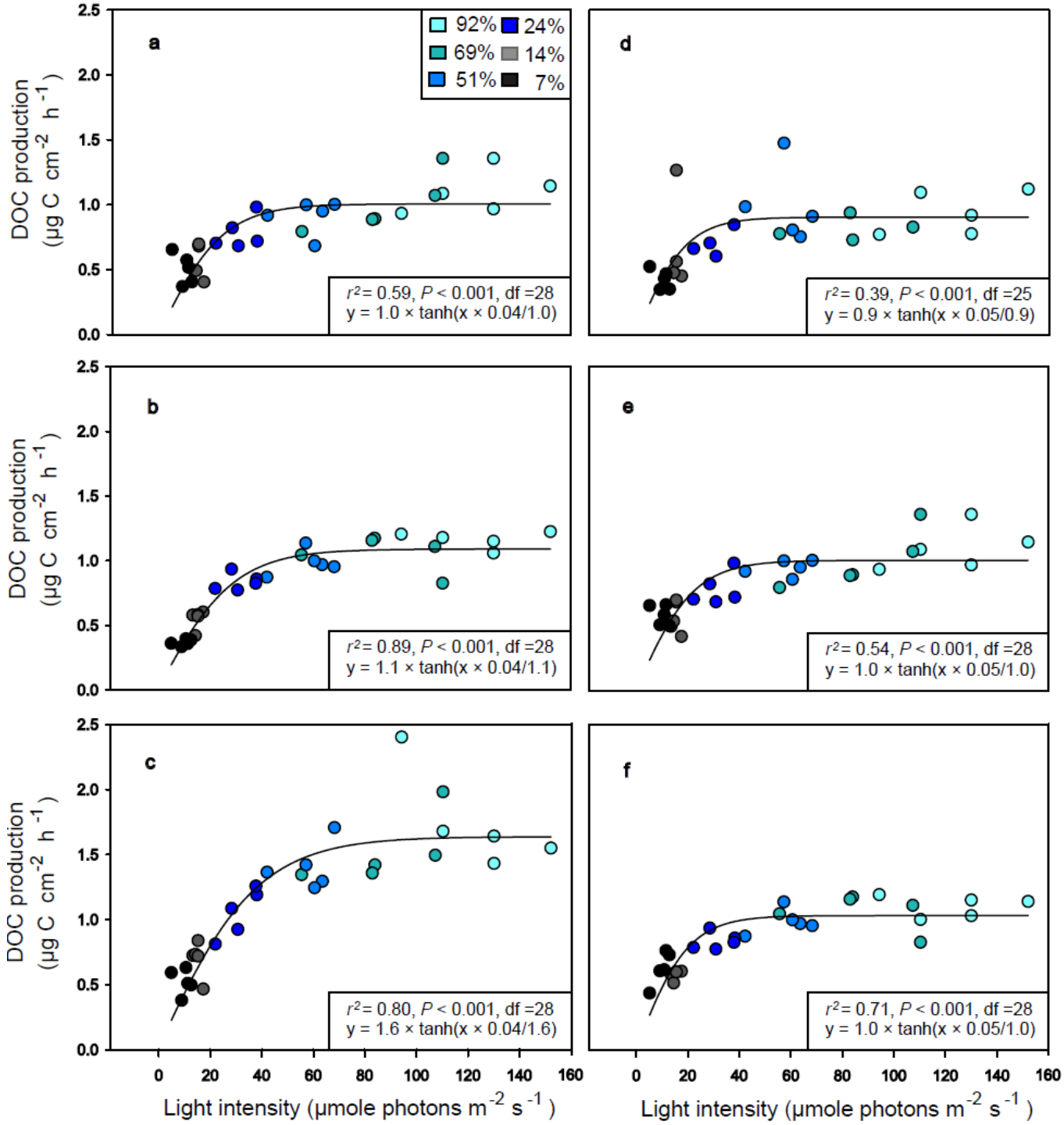
Parameter	**7%T**	**14%T**	**24%T**	**51%T**	**69%T**	**92%T**
PO_4 $t_{6h(day)}$ ($P\text{-}PO_4$ µg cm^{-2})	0.5±0.1 (0.0 to 0.9)	0.4±0.1 (0.0 to 0.6)	0.2±0.1 (0.0 to 0.5)	0.1±0.0 (0.0 to 0.3)	0.1±0.0 (0.0 to 0.3)	0.1±0.0 (0.0 to 0.6)
PO_4 $t_{6h(night)}$ ($P\text{-}PO_4$ µg cm^{-2})	0.6±0.1 (0.2 to 0.9)	0.4±0.1 (0.0 to 0.8)	0.2±0.1 (0.0 to 0.5)	0.1±0.0 (0.0 to 0.5)	0.1±0.0 (0.0 to 0.2)	0.0±0.0 (0.0 to 0.1)
PO_4 $flux_{(day)}$ ($P\text{-}PO_4$ µg cm^{-2})	0.3±0.0 (0.1 to 0.5)	0.4±0.0 (0.3 to 0.5)	0.6±0.1 (0.3 to 0.9)	0.7±0.1 (0.5 to 1.1)	0.8±0.1 (0.5 to 1.1)	0.7±0.1 (0.2 to 1.1)
PO_4 $flux_{(night)}$ ($P\text{-}PO_4$ µg cm^{-2})	0.3±0.0 (0.2 to 0.6)	0.4±0.1 (0.2 to 1.0)	0.7±0.1 (0.4 to 1.0)	0.8±0.1 (0.5 to 1.2)	0.8±0.1 (0.5 to 1.2)	0.9±0.1 (0.5 to 1.2)
NO_3 $t_{6h(day)}$ ($N\text{-}NO_3$ µg cm^{-2})	13.2±1.1 (8.5 to 18.1)	12.3±1.1 (8.1 to 17.0)	11.6±1.0 (8.1 to 16.1)	10.6±1.0 (6.8 to 14.8)	10.0±0.9 (6.1 to 14.1)	9.3±0.9 (0.02 to 13.3)
NO_3 $t_{6h(night)}$ ($N\text{-}NO_3$ µg cm^{-2})	15.5±1.3 (10.3 to 20.6)	14.8±1.3 (9.7 to 20.2)	15.1±1.3 (10.1 to 20.1)	14.7±1.3 (10.0 to 19.7)	14.6±1.2 (10.0 to 19.3)	14.3±1.2 (9.6 to 18.9)
NO_3 $flux_{(day)}$ ($N\text{-}NO_3$ µg cm^{-2})	1.0±0.2 (-0.3 to 2.2)	1.6±0.1 (0.8 to 2.3)	2.7±0.3 (1.5 to 3.9)	3.6±0.4 (2.2 to 5.7)	4.2±0.5 (2.3 to 6.9)	4.9±1.1 (1.9 to 17.7)
NO_3 $flux_{(night)}$ ($N\text{-}NO_3$ µg cm^{-2})	0.0±0.2 (-1.5 to 0.6)	0.3±0.3 (-1.5 to 3.4)	0.5±0.2 (-1.1 to 1.4)	0.8±0.2 (-0.8 to 2.6)	0.9±0.2 (-0.4 to 2.3)	1.2±0.2 (-0.3 to 2.0)



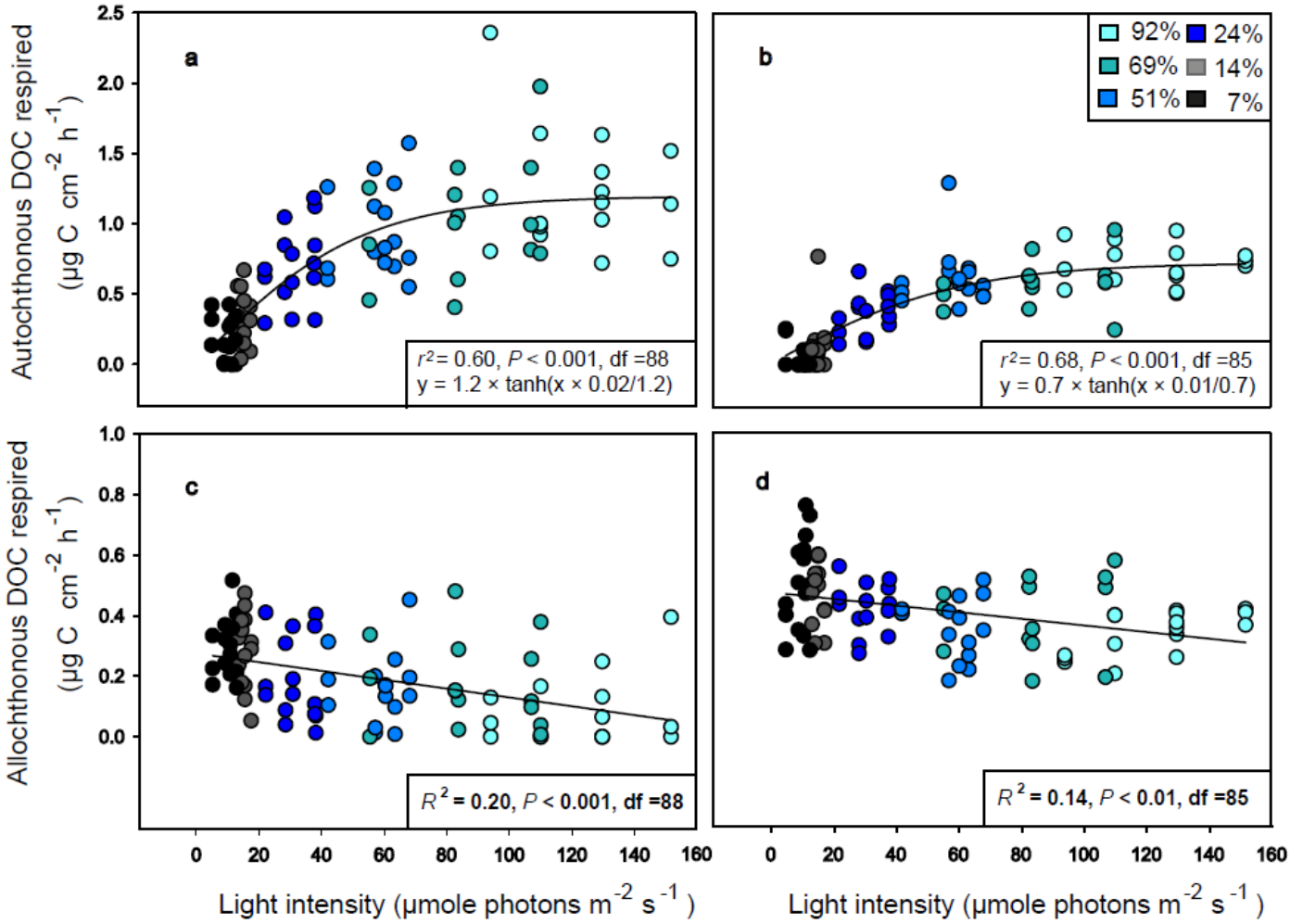
SI Figure 1. Three fluorescent components were modeled by parallel factor analysis (PARAFAC) from excitation emission matrices. The first component was assigned as humic-like (a) and the other two components were assigned as amino acid-like (b-c).



SI Figure 2. Mean ± standard deviation and non-linear regression analyses of chlorophyll *a* (a), cell abundance (b) and biofilm biomass (c) across the light gradient (% transmission of the incident light (%T)) after 8 h (circles) and 128 h (squares).



SI Figure 3. Non-linear regression of the C mass balance (C_{budget}, µg C cm^{-2} h^{-1}) in the microcosms across the light gradient (% transmission (%T) of the incident light) during day-time (a-c; 32, 56 and 80 h) and night-time incubations (d-f; 20, 44 and 68 h).



SI Figure 4. Allochthonous and autochthonous DOC contributing to community respiration across the light gradient (% transmission (%T) of the incident light) for day-time (a, c) and night-time (b, d) incubations.

7. Discussion

7.1 Priming effects in freshwater ecosystems

Though priming is a well-established mechanism in soil sciences, evidence for priming in aquatic ecosystems remains very limited yet. The mechanisms involved in priming are not well understood, though they are believed to be largely controlled by energy and nutrient availability as well as by stoichiometric constraints (Guenet et al. 2010). We were not able to detect increased mineralization rates of refractory carbon upon the addition of labile carbon whether in hyporheic stream microbial communities (Bengtsson et al. 2014) or in benthic biofilm communities (Paper III). This lack of priming may likely be explained by the fact that the labile carbon sources were primarily used for biofilm metabolism (gross primary production and respiration) and growth (Paper II & III) and not for the production of extracellular enzymes like phenol oxidase for refractory DOM degradation. The hyporheic microbial communities exhibited similar phenol oxidase expression levels with and without primer addition (Paper I), which may be explained by the fast uptake of labile DOM by hyporheic heterotrophs at the bioreactor inflow and longer residence times of more refractory DOM in the bioreactors (Kaplan et al. 2008) (Paper I). In fact the majority of the hyporheic community may not have encountered labile DOM, which is furthermore supported by rather low levels of leucine-aminopeptidase and beta-glucosidase activities (Paper I). In contrast, the clear gradient of phototrophic primary production exhibited by the benthic biofilms exposed the microbial heterotrophs to different levels of algal exudates. Rather than showing enhanced use of refractory DOM under high light, the biofilm communities adapted their use of allochthonous DOM depending on the presence of autochthonous DOM sources (Paper II & III). This preferential use of autochthonous DOM over allochthonous DOM is furthermore supported by the fact that we did not observe co-metabolism strategies in the biofilm communities (Blagodastkaya and Kuzyakov 2008).

The breakdown of refractory DOM can liberate limiting nutrients, rendering priming a potential nutrient mining strategy under nutrient limiting conditions (Guenet et al. 2010, Kuzyakov et al. 2010). On the other hand, addition of inorganic nutrients has been shown to enhance priming (Carlson et al. 2002). Here, nutrient (N and P) additions in combination with a labile primer actually led to a slight decrease in refractory DOM degradation by hyporheic microbial communities (Bengtsson et al. 2014), which contrasts findings from marine microbial communities (Carlson et al. 2002). Similarly, benthic biofilms growing under P limiting conditions showed no indications of priming. Instead, high availability of autochthonous carbon led to an increase in phosphatase activity and enhanced degradation of algal exudates without the increased use of allochthonous DOM sources (Paper II&III). This is consistent with findings from other stream biofilms, where nutrient limiting conditions led to increased algal-bacterial interactions and subsequently higher internal cycling of algal exudates within biofilms (Ziegler et al. 2009, Ziegler et al. 2010). Though the release of nutrients from refractory DOM appears to be an important mechanism in soils (e.g. Blagodadskaya 1998, Raynaud et al. 2006), it seems of minor importance for stream biofilm communities. The occurrence of priming may depend on the composition and concentration of labile DOM (Guenet et al. 2010, Smith et al. 2007). However, I found no effect of labile substrate complexity (algal exudates versus glucose) on refractory DOM degradation (Paper I).

7.2 Effects of labile and refractory DOM on biofilm structure

The most abundant taxonomic groups in this study were Proteobacteria, Actinobacteria, Bacteriodetes, Gemmatimonadetes, Verrucomicrobia and Planctomycetes (Paper I&II). These phyla have frequently been found to constitute the most important microbial groups in freshwater biofilms (e.g. Eiler et al. 2004, Romani et al. 2014, Besemer et al. 2012). Alpha-Proteobacteria was the most abundant class in the hyporheic and benthic biofilms (Paper I &

II), which is consistent with earlier findings for freshwater biofilms (Hall et al. 2012, Lear et al. 2009, Romani et al. 2014). This can likely be explained by the ability of Alpha-Proteobacteria to degrade humic substances (Newton et al. 2011) as the refractory allochthonous DOM source used in all our experiments was characterized by a high degree of humification (Paper III). This may also have fostered the high abundance of Bacteriodetes in this study (Paper I & II). This phylum is known to degrade complex macromolecules and increase in abundance when labile DOM is depleted (Knoll et al. 2001, Bartrons et al. 2012). Furthermore, Bacteriodetes have been associated with cyanobacterial blooms (Newton et al. 2011). We detected a considerable number of Cyanobacteria in our benthic biofilms that increased in relative abundance along the light gradient (Paper II), but Bacteriodetes did not exhibit any clear trends. Interestingly, Acidobacteria were a dominant phylum in the hyporheic biofilms, whereas they were of minor importance for the benthic biofilms.

Though, hyporheic and benthic, active and bulk biofilm communities harbored similar phyla, DOM resource use had different effects on community composition (Paper I&II). Chemical composition of DOM seemed to be a strong driver of community composition in the hyporheic biofilms as allochthonous DOM drove shifts in the composition of the hyporheic microbial community, whereas autochthonous DOM affected community composition only transiently (Paper I). Benthic biofilm community composition was strongly influenced by light availability and the availability of different DOM resources (Paper II&III). Consistent with our findings, studies on experimental stream biofilms observed a strong effect of light on community composition, but no consistent effect on diversity (Romani et al. 2014). Our richness estimates from the hyporheic biofilm communities coincide well with richness measures from hyporheic biofilms in boreal streams (1000-1300 OTUs, Besemer et al. 2012) and experimental hyporheic biofilms (600-2300 OTUs, Hall et al. 2012). We found far higher richness in the hyporheic biofilms (820-1000 OTUs) than in the benthic biofilm communities (320-410 OTUs), probably due to the heterogeneity of stream sediments in terms of resource

gradients and the available niches (Brunke and Gonser 1997, Torsvik et al. 2002). The availability of different resources may decrease inter-specific competition, thereby supporting the diversity of the microbial community (Hand.2015, Zelezniak et al. 2015, Burgos-Caraballo et al. 2014). In contrast, heterotrophs in benthic, mixed algal-bacterial communities primarily used autochthonous or allochthonous DOM sources depending on light intensity (Paper III). Therefore, this environment may support less community specialization and niche differentiation than hyporheic biofilms, leading to lower community diversity. Furthermore, the bulk community showed a clearly different community composition and higher alpha-diversity than the active community in hyporheic biofilms. This suggests that the bulk community provides a seed bank of dormant organisms, which prevents the extinction of rare taxa (Jones and Lennon 2010).

7.3 Effects of labile and refractory DOM on biofilm function

To understand the fundamental mechanisms that drive priming effects, it is important to investigate the influence of resource availability on microbial community structure and its linkage to community function. Consistent with another study (Frossard et al. 2012), we found that resource availability influenced community composition in hyporheic bacterial communities, but bulk community composition and diversity did not affect community function, indicative of functional redundancy (Paper I). In benthic biofilms we found that resource availability strongly affected multifunctionality and that bacteria under low light availability were unable to maintain multifunctionality suggesting a limited degree of functional redundancy (Paper II). Benthic biofilm diversity was decoupled from multifunctionality across the whole light gradient (Paper II). This contrasts studies of lake (Peter et al. 2011) and stream bacterial communities (Ylla et al. 2013) that reported a negative effect of bacterial diversity loss on community multifunctionality. As diversity was generally high in our stream biofilms, functional diversity of the microbial community may be more

important for maintaining ecosystem processes than their taxonomic diversity (Lecerf and Richardson 2010, Tilman et al. 1997). These findings have shown that resource availability has important implications for the cycling of allochthonous and autochthonous DOM within stream biofilms and can determine whether biofilms act as a sink or source of carbon.

7.4 Potential reasons for the absence of priming in stream biofilms

Both temporal and spatial aspects of microbial communities may foster priming, as increased residence times of substrates and spatial arrangements within biofilms influence microbial community structure and function. Priming studies in aquatic ecosystems show similar incubation times (7 to 45 days) than our studies (e.g. Danger et al. 2013, Catalan et al. 2015, Kuehn et al. 2014, Guenet et al. 2014, VanNugteren et al. 2009). Therefore, the occurrence of priming seems to not exclusively be a function of exposure time of the biofilm community to labile and refractory DOM. We expected to observe priming effects due to the spatial proximity between auto- and heterotrophs in benthic biofilm communities or as a consequence of the pulsed availability of labile carbon in hyporheic microbial communities. However, in our stream biofilms, bacterial cells used labile carbon sources for growth and biomass accrual instead of investing it into extracellular enzyme production that is required for recalcitrant carbon degradation. Biofilm microbes are embedded in an EPS matrix receiving temporarily labile DOM from algae and terrestrial, refractory DOM at a steady rate as the biofilm matrix buffers environmental fluctuations in substrate supply (Besemer et al. 2012). Therefore, heterotrophs may adapt their use of allochthonous DOM depending on the availability of labile DOM. Clear diurnal patterns could be observed in the use of allochthonous DOM by the benthic biofilm community, though labile carbon shortage never lasted longer than 10 hours. In contrast, in soils labile carbon pulses just occur seasonally and are spatially constrained (Jobbagy and Jackson 2000). This renders priming an important strategy for soil microbes to endure labile carbon shortage. Studies that reported priming

effects in aquatic ecosystems were systems dominated by leaf litter breakdown (Kuehn et al. 2014, Danger et al. 2013), where algal exudates stimulated microbial heterotrophs, especially fungal decomposers. This supports the important role of fungi in refractory DOM degradation (Carney et al. 2007). Also nutrient mining strategies were found in these systems as increased leaf-litter break down occurred under low nutrient conditions (Danger et al. 2013).

In conclusion, priming of refractory DOM by labile algal exudates in stream biofilms seems to not be the major process leading to the net-heterotrophy of stream ecosystems, rather enhanced growth and biomass build up by the use of labile carbon sources seems to explain increased biofilm respiration rates. During the day or high light availability microbial respiration was mainly fueled by autochthonous DOM sources, whereas refractory DOM use was enhanced at night or low light availability. These findings have important implications for stream ecosystems as increased carbon loading from soils may increase water turbidity and thereby enhance allochthonous DOM degradation. Future research should thus aim to investigate which specific conditions may foster priming in freshwaters, as this mechanism seems to not be as prevalently occurring as in terrestrial ecosystems.

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9. Zusammenfassung

Fluss Ökosysteme zeichnen sich durch hohe biogeochemische Aktivität aus, was zu ansteigendem CO_2 Ausstoß an die Atmosphäre führt. Es wurde prognostiziert, dass der Klimawandel zu erhöhtem Transport von terrestrischem Kohlenstoff in Inlandgewässer führt, was wiederum vermehrte Treibhaus-Gas-Emission aus Flüssen an die Atmosphäre zur Folge hat und somit die globale Erderwärmung fördert. Deshalb ist es wichtig zu erforschen wie dieser terrestrische Kohlenstoff in Flüssen durch mikrobielle Gemeinschaften abgebaut wird. Es wird generell angenommen, dass terrestrischer Kohlenstoff eine geringe Bioverfügbarkeit aufweist. Studien in Böden haben jedoch gezeigt, dass das Vorhandensein von labilen Kohlenstoffverbindungen die Mineralisation von refraktärem Kohlenstoff durch den so-genannte Priming-Effekt fördern kann. In Fluss-Biofilmen, welches die vorherrschende Form von mikrobiellem Leben in Flüssen darstellt, koexistieren phototrophe und heterotrophe Mikroorganismen in unmittelbarer Nähe zueinander. Die Freisetzung von photosynthetisch hergestellten, labilen Kohlenstoffverbindungen könnte daher den Abbau von refraktärem, terrestrischem Kohlenstoff durch Priming anregen und in Folge zu erhöhter CO_2 Emission aus Flüssen beitragen. Meine Doktoratsarbeit hat sich mit der Erforschung von Priming-Effekten in benthischen und hyporheischen Flussbiofilmen beschäftigt, da Priming ein potentiell wichtiger Mechanismus zum Abbau von terrestrischem Kohlenstoff in Flüssen sein könnte. Meine Forschungsarbeit mit Fluss-Biofilm-Gemeinschaft konnte Priming nicht als wichtigen Mechanismus zum Abbau von terrestrischem Kohlenstoff in Fluss-Ökosystemen bestätigen. Die Energie, die aus dem Abbau von labilem Kohlenstoff gewonnen wurde, wurde nicht für die Synthese von extrazellulären Enzymen und den nachfolgenden Abbau von refraktärem Kohlenstoff durch die Biofilm-Gemeinschaft genutzt. Dies zeigt, dass Ko-Metabolismus Strategien in diesen Biofilmen nicht von Bedeutung waren. Die Absenz von Priming könnte durch den bevorzugten Abbau von labilem Kohlenstoff gegenüber refraktärem Kohlenstoff

durch die mikrobielle Gemeinschaft erklärt werden. Andererseits konnte ich jedoch Effekte von labilem und refraktärem Kohlenstoff auf die Zusammensetzung und Funktion von Biofilmen zeigen, aber nicht auf deren Vielfalt. In hyporheischen Biofilmen hat refraktärer Kohlenstoff zu einer Veränderung der mikrobiellen Zusammensetzung geführt, wobei labiler Kohlenstoff die Zusammensetzung nur kurzfristig beeinflusst hat. Beide Kohlenstoff-Quellen haben die Biofilm Funktion nicht beeinflusst, was auf eine funktionelle Redundanz der Gemeinschaft hinweist. Temporäre Veränderungen in der Zusammensetzung der aktiven, hyporheischen Biofilm-Gemeinschaft war durch eine Veränderung der Funktion begleitet. In benthischen Biofilmen hatte Licht einen starken Einfluss auf Primärproduktion und phototrophe Kohlenstoff-Freisetzung, welches die Zusammensetzung des Kohlenstoffes, der für heterotrophes Wachstum zur Verfügung stand, stark veränderte. Dies beeinflusste die Zusammensetzung der mikrobiellen Gemeinschaft und hatte ebenfalls einen starken Einfluss auf die Biofilm Funktion. Spezifische extrazelluläre Enzym-Aktivitäten waren stark mit Nährstoff-Abbau und mikrobieller Respiration korreliert, welches den Link zwischen der taxonomischen Biofilm-Zusammensetzung, deren Funktion und biogeochemischen Stoff-Flüssen unterstreicht. Generell war die Biofilm Vielfalt nicht erhöht oder erniedrigt nach der Zugabe von labilem Kohlenstoff, jedoch zeigten hyporheische Biofilme eine weit höhere Vielfalt als benthische Biofilme. Dies könnte auf die Ressource-Gradienten in hyporheischen Biofilmen zurück zu führen sein, welche eine erhöhte Substrat-Spezialisierung der mikrobiellen Gemeinschaft und somit verminderte Konkurrenz um dieselben Kohlenstoffquellen zur Folge hatten. Zusammenfassend stimmen meine Forschungsergebnisse der minderen Bedeutung von Priming in hyporheischen und benthischen Biofilmen mit anderen Studien überein, die Priming ebenfalls nicht als universell auftretenden Mechanismus in mikrobiellen Gemeinschaften in Inlandgewässern bestätigen konnten.

10. Curriculum Vitae

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Education:

2010 to present: PhD in Biology, **Dissertation Research**: "*Are stream biofilms a prime site for priming?*"
Supervisor: Prof. Tom Battin, University of Vienna, Faculty of Life Sciences, Althanstraße 14, 1090 Vienna, Austria

2009 to 2010: MSc. in Molecular Biotechnology, **Thesis Research**: *"Transcriptional study of Oenococcus oeni adaptation to wine stress related conditions"*
Supervisor: Prof. Christina Reguant and Prof. Kuen-Krismer. Universitat Rovira i Virgili, Biotechnology Department, 43007 Tarragona, Spain

2006 to 2010 Msc. studies in Molecular Biotechnology at the University of Applied Sciences, Campus Vienna Biocenter, 1030 Vienna, Austria

Positions:

August 2011 to present: **PhD student** in Biology in the research group of Prof. Tom Battin, University of Vienna, Faculty of Life Sciences, Althanstraße 14, 1090 Vienna, Austria

September 2012 to December 2012 and September 2013 to November 2013: **Guest researcher**: "Meta-proteomics of bacterial stream biofilms including data analyses" in the research group of Prof. Katharina Riedel, Department of Microbiology, Ernst Moritz Arndt University of Greifswald, 17489 Greifswald, Germany

May 2012: **Guest researcher**: "Extracellular enzymatic activity assays of bacterial stream biofilms including data analyses" in the research group of Prof. Anna Romani, University of Girona, Department of Aquatic Ecology, Campus Montilivi- 17071 Girona, Spain

October 2010 to July 2011: **Chemical technical assistant** in the research group of Prof. Tom Battin, University of Vienna, Faculty of Life Sciences, 1090 Vienna, Austria.

Publications:

Wagner K, et al. 2015. Light availability affects stream biofilm bacterial community composition and function, but not diversity. Microbial Ecology: doi:10.1111/1462-2920.12913

Wagner K, et al. 2014. Functional and structural responses of hyporheic biofilms to varying sources of dissolved organic matter. Appl. Environ. Microbiol:doi:10.1128/AEM.01128-14

Bengtsson MM, **Wagner K**, et al. 2014. No evidence of aquatic priming effects in hyporheic zone microcosms. Scientific Reports:doi:10.1038/srep05187

Publications in submission:

Wagner K, et al. 2015. Light intensity mediates a shift from allochthonous to autochthonous carbon use in stream biofilms. Limnology and Oceanography

Teaching:

2015: **Lecturer**: "Aquatic Microbial Ecology - Introduction to Methods", University of Vienna
2015: **Tutor**: "Aquatic Microbial Ecology - Introduction to Methods", University of Vienna

First Author Presentations at Professional Meetings and Seminars:

2014: **Wagner K**, Bengtsson MM, Besemer K, Sieczko A, Burns NR, Herberg ER and Battin TJ. *"Functional and structural responses of stream biofilms to varying resources"* **poster presentation**, Biofilms6 conference, Vienna, Austria

2013: **Wagner K**, Bengtsson MM, Burns NR, Herberg ER, Wanek W, Kaplan L, Van Oevelen D, and Battin TJ. *Interactions between recalcitrant and labile organic carbon in streams: Can stream biofilms mediate priming effects?* **oral presentation,** Aquatic Microbial Ecology Conference, Stresa, Italy

2013: **Wagner K**, Bengtsson MM, Burns NR, Herberg ER and Battin TJ. *"Meta-genomic study of bacterial freshwater biofilms"* **oral presentation** in the seminar series of the Department of Microbiology, University of Greifswald, Germany

2012: **Wagner K**, Herberg ER, Bengtsson MM, Sieczko A, Burns NR and Battin TJ. *"Extracellular enzyme activity assays (EEA) as a tool to investigate priming in freshwater biofilms"* **poster presentation**, International Symposium of Microbial Ecology, Kopenhagen, Denmark

2012: **Wagner K**, Sieczko A, Bengtsson MM, Burns NR, Herberg ER and Battin TJ. *"The importance of priming effects in bacterial freshwater biofilms"* **oral presentation** in the seminar series of the Department of Biology, University of Girona, Spain

2012: **Wagner K**, Sieczko A, Bengtsson MM, Burns NR, Herberg ER and Battin TJ. *"Extracellular enzyme activity assays (EEA) as a tool to investigate priming in freshwater biofilms"* **poster presentation**, European Geosciences Union General Assembly, Vienna, Austria

Grants:

2012: Research grant from the University of Vienna to **Wagner K** to conduct a meta-proteome study of bacterial stream biofilms at the University of Greifswald, Germany