Response of biofilm-dwelling ciliate communities to enrichment with algae

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ABSTRACT: The role of benthic versus planktonic algae in shaping semi-natural ciliate biofilms was studied using miniature flow cells fed by a permanent flow of natural field water from the River Rhine, Germany. Increased food availability for the ciliates was achieved either by supplementation with planktonic algae or by artificial illumination (to stimulate benthic algal growth). Manipulation of both resources significantly increased the total abundance of ciliates and caused significant shifts in the structure of the ciliate community. Supplementation with planktonic algae particularly stimulated suspension-feeding Peritrichia, whereas Choreotrichia were reduced, probably due to indirect effects such as competition or interference. Increased density of benthic algae especially stimulated surface-feeding groups (e.g. Stichotrichia, Nassophorea), causing the establishment of seasonally atypical structures in the ciliate community, as has been observed in late fall. Ciliates generally responded faster to increased resources during the summer, as compared with winter, suggesting that temperature, as well as the availability of resources, has an important role in shaping ciliate biofilms. Our results are among the first that demonstrate the different contributions of planktonic and benthic algae in shaping ciliate biofilms. Furthermore, they accentuate the need for studies with a higher taxonomic resolution when addressing the responses of microbial biofilms to the increased availability of food.

KEY WORDS: River biofilm · Benthic ciliates · Bottom-up · Grazing · Periphyton

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INTRODUCTION

Heterotrophic protozoans can occur in high densities within microbial biofilms, where they can alter both the structure and function of bacterial biofilms (Weitere et al. 2005, Wey et al. 2008, Böhme et al. 2009). In terms of biomass, microbial freshwater biofilms are often dominated by ciliates that can form complex communities of many different taxonomic and functional types (Arndt et al. 2003, Parry 2004, Gong et al. 2005). As inhabitants of the sediment–water interface, ciliates can potentially prey on both surface-attached prey (e.g. Weitere et al. 2005, Königs & Clevén 2007) and suspended prey (e.g. Eisenmann et al. 2001,

Posch et al. 2001, Weitere et al. 2003). While these functionally different ciliates were found to coexist within biofilms (e.g. Franco et al. 1998, Parry 2004, Gong et al. 2005, Primc-Habdija et al. 2005), the contribution of planktonic versus benthic resources in shaping the structure of ciliate biofilms is not clearly understood. Experimental bottom-up manipulations of biofilms in the field are commonly accomplished by the addition of nutrients, which results in rather unspecific stimulation of several microbial groups, such as bacteria, microalgae, and ciliates. For example, the addition of inorganic nutrients to lake mesocosms has been shown to stimulate bacterial, algal, and ciliate biomass (Hillebrand et al. 2002, Wickham et al. 2004). Similarly,

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Greenwood et al. (2007) have shown that whole-stream fertilization results in a general increase in microbial activity. However, little is known on how different resources (suspended, and surface-attached pico- and nano-sized particles) contribute to shaping the structure of ciliate biofilms.

Norf et al. (2007) have developed a flow-cell system for growing and experimentally manipulating microbial biofilms under semi-natural conditions. These flow cells are fed by a permanent current of field water that contains both the organisms for biofilm colonization and the suspended resources necessary for biofilm development. Using increments of benthic and planktonic bacteria, it was shown that the addition of planktonic bacteria can strongly stimulate ciliate communities, whereas enhanced growth of benthic bacteria results in the development of grazing-resistant filamentous bacteria (Queck et al. 2006, Norf et al. 2009a). Such defense strategies against protozoan grazers are also common in benthic microalgae, e.g. by attaining large cell sizes or by the secretion of an extracellular matrix (Barranguet et al. 2005). Nevertheless, increased bacterial biomass can accelerate biofilm colonization by ciliates and can alter the structure of the ciliate community. However, early and late succession stages within a developing biofilm can clearly differ in their functional composition, i.e. smaller bacterivorous ciliates, such as *Hymenostomatia*, colonize biofilms earlier than is usually the case with algivorous ciliates such as large *Peritrichia* and *Stichotrichia*. This could mean that, while algal supplements probably do not affect early biofilm development, they may influence the structure of the ciliate community in maturing biofilms.

In the present study, the effects of increased densities of suspended and benthic algae on the development of ciliate biofilms were tested using miniature flow cells. We checked for both quantitative and qualitative (i.e. community structure) effects of the increased availability of resources on ciliate biofilms. The experiments were performed repeatedly during different times of the year (i.e. summer, fall and winter), incorporating different background conditions with respect to ambient resource load and water temperature.

MATERIALS AND METHODS

Study site and facilities. All experiments were conducted aboard the Ecological Rhine Station of the University of Cologne, Germany. This station is a ship that is permanently anchored in the main stream of the River Rhine in Cologne at Rhine-km 684.5, which refers to the distance from Lake Constance, the source of the non-alpine Rhine. The ship contains laboratories equipped with pump systems that continually supply

the laboratories with unaltered field water from the River Rhine. The field water contains both the source organisms for biofilm colonization (e.g. unicellular algae, bacteria, heterotrophic flagellates, ciliates) and the suspended resources essential for biofilm development.

Experimental setup. The flow cells used for the experiments were modified from flow cells commonly used in laboratory experiments (e.g. Stoodley & Woodward 2003), as described by Norf et al. (2007). The flow cells allowed us to examine living biofilm communities by light microscopy and also to control the manipulation under semi-natural conditions without disturbing the biofilms. The inner dimensions of these flow cells are: $40 \times 19 \times 5$ mm (length $\times$ width $\times$ height), enclosing a volume of 3.8 ml. The flow cells were fed with a constant flow-through of River Rhine water (1 volume exchange min^{-1}) using tube pumps. The ambient temperature of the field water, which was measured constantly with a digital thermometer, was maintained by storing the flow cells in a temperature-controlled pond set up in a fenestrated room that allowed $\sim 1.5\%$ of the natural light intensity to reach the flow cells.

Two types of resource treatment were applied in order to distinguish between the effects of increased densities of planktonic and benthic algae on the succession of organisms in ciliate biofilms (Fig. 1); in this approach, the experimental manipulation of resources and the culturing of biofilms started simultaneously. The abundances of planktonic bacteria and algae within the field water were determined, by microscopy, from counts of 4',6-diamidino-2-phenylindole (DAPI)-stained and Lugol-fixed samples, respectively. The temperature was measured constantly using a digital thermometer (see Table 1 for data). Each treatment consisted of 4 replicates, except in Expt Pla1, which only consisted of three replicates. In Expt Pla2, one of the four replicates failed.

The first type of manipulation was designed to test the effects of enrichment with planktonic algae (type Pla; Fig. 1a) on the development of the ciliate biofilm at high ambient water temperatures (Expt Pla1, see Table 1) and at low ambient water temperatures (Expt Pla2, see Table 1), i.e. during summer and winter, respectively. For this purpose, a suspension of freeze-dried green algae (*Chlorella* sp.) was fed to the flow cells (Pla ENR). The suspension was prepared by suspending 0.5 g of *Chlorella* powder in 15 ml particle-free filtered ($0.2 \mu\text{m}$) field water. Algal aggregates were removed by filtering the suspension through a $20 \mu\text{m}$ filter cartridge. The algal cells in the resulting filtrate had a mean diameter of $5 \mu\text{m}$. A cell density of 3×10^6 cells ml^{-1} was obtained by diluting the algal suspension with particle-free field water. The algal suspension was added to the flow cells through silicone

hoses (not shown). Because the ratio of algal suspension to field water was 1:100, the final concentration of supplemented algae was 3×10^4 cells ml^{-1} in the flow cells. As a control, an equal amount (0.04 ml min^{-1}) of algal suspension was filtered through a $1.2 \mu\text{m}$ filter cartridge (in order to remove all suspended algal cells) and fed to the flow cells (hereafter: Pla CON); this treatment was designed to check for possible effects of bacteria that developed within the suspension and of dissolved organic matter (DOM) leaching from the dead algal cells that could potentially enhance bacterial growth within the flow cells.

The second type of resource manipulation was designed to investigate the effects of an increased density of benthic algae (type Ben; Fig. 1b) on the development of ciliate biofilms at moderate water temperatures (Expt Ben1, Table 1) and at low water temperatures (Expt Ben2, Table 1), i.e. during fall and winter, respectively. For this purpose, the flow cells were stored in a temperature-controlled pond (adjusted to ambient water temperature) that also included artificial illumination devices (light intensity: $33 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) with a light:dark cycle of 12:12 h (hereafter: Ben LIGHT). Treatments run in complete darkness, but with otherwise unaltered ambient conditions (hereafter: Ben DARK), served as a direct control of the Ben LIGHT treatments.

Fig. 1. Experimental setups (schematic). (a) Type Pla, i.e. an approach designed to test the effects of the supplementation of planktonic algae (*Chlorella* sp.) on the development of biofilm-dwelling ciliate communities. Pla ENR = flow cells enriched with *Chlorella* suspension; Pla CON = control for Pla ENR, flow cells fed with algae-free filtrate ($1.2 \mu\text{m}$) of the suspension. (b) Type Ben, i.e. an approach designed to test the influence of light-stimulated growth of benthic algae on the development of biofilm-dwelling ciliate communities. Ben LIGHT: illuminated flow cells containing otherwise untreated field water from the River Rhine; Ben DARK: flow cells in complete darkness containing otherwise untreated field water

Expts Ben2 and Pla2 were conducted simultaneously and were thus under the same ambient conditions.

Analysis of ciliate biofilms by microscopy. The ciliate biofilms cultivated within the flow cells were analyzed by putting the flow cells directly onto the cross table of a Zeiss Axioskop light microscope equipped with phase contrast. The first survey was performed 24 h after starting the experiments and was repeated every 1 to 3 d until the end of the experiments (Pla1: 12 d; Pla 2: 20 d; Ben1: 16 d; Ben2: 24 d). The surveys comprised both quantitative (abundance) and qualitative (i.e. species composition) analyses. The structure of the ciliate community was documented by video recordings for later identification of species not instantly recognized. Ciliates were quantified at a magnification of 100 to $200\times$ (up to $1000\times$ for identification of species) in defined areas that were randomly distributed over the complete biofilm surface area using an eyepiece grid. At least 60 ciliates per flow cell were recorded. Ciliates were identified using general identification keys (Foissner et al. 1991, 1992, 1994, 1995, Foissner & Berger 1996), following the classification scheme of Adl et al. (2005). Heterotrophic flagellates (HFs) were quantified in all experiments, and surface-attached microalgae were quantified in both Ben experiments, following the same procedure as applied to the ciliates. The abundances of the HFs were tracked because they could be (1) indicators for indirect effects of resources, due to stimulated bacterial growth, and (2) a potential food source for particular ciliates.

Statistical analyses. Statistical analyses were performed with SPSS 15.0 for Windows. For quantitative comparisons between the treatments, repeated measures ANOVAs (rmANOVAs) were calculated using 'time' as the inner-subject factor and 'treatment' (i.e. resource enrichment) as the between-subject factor. Using rmANOVAs, the effects of increased availability of resources on both the total ciliate abundance and the abundance of particular taxonomic units (fourth to fifth rank) of the ciliates were tested. The first days of biofilm succession, during which only very low numbers of ciliates were observed (Expt Pla1: Days 1 to 3; Expt Ben1: Days 1 to 4; Expts Pla2 and Ben2: Days 1 to 7), were excluded from the calculations in order to reduce noise from the observation data. Furthermore, we considered only those taxonomic units that contributed at least 10% of the total ciliate abundance in any of the treatments during the whole experiment. All data were log-transformed in order to achieve homogeneous variances. In the case of multiple comparisons, the Dunn–Sidak correction method was applied in order to control the familywise Type I error rate.

The effects of the availability of resources on the structure of the ciliate communities were analyzed by

calculating the Bray–Curtis similarity index (Bray & Curtis 1957) for the final day of the experiments using Primer 6.0 software (Primer-E). Data were square-root-transformed in order to reduce the probability of artifacts due to highly dominant ciliate groups. The similarity indices obtained were plotted 2-dimensionally using non-metric, multi-dimensional scaling (NMDS). Significant differences in the structures of the ciliate community between all replicates were determined by cluster analyses using a group average algorithm and adjacent SIMPROF test ($\alpha < 0.05$) and were indicated graphically in the NMDS plots.

RESULTS

Water temperatures and planktonic abundances of microbes

Mean water temperatures in the River Rhine were 20, 15, and 8°C during Expts Pla1 (summer), Ben1 (fall) and Pla2/Ben2 (winter), respectively (Table 1). Plankton densities, and thus the availability of natural resources for the developing ciliate biofilms, showed large variations with respect to the date of the experiment. In summer (Expt Pla1), the abundances of planktonic algae (420 ± 110 cells ml⁻¹) and bacteria ($1.6 \pm 0.2 \times 10^6$ cells ml⁻¹) were highest (Table 1). In Expts Ben1 and Pla2/Ben2, the abundances of planktonic algae were 180 ± 20 and 150 ± 20 cells ml⁻¹, respectively. Planktonic bacteria showed values of $1.1 \pm 0.1 \times 10^6$ (Ben1) and $1.4 \pm 0.2 \times 10^6$ cells ml⁻¹, respectively.

The abundance of HFJs was tracked in all experiments. However, neither stimulation of benthic algae (Ben LIGHT) nor supplementation with planktonic algae (Pla ENR) showed significant effects on the abundance of the HFJs (Fig. 2; resource effects in rmANOVA: Expt Pla1, $p = 0.976$; Expt Pla2, $p = 0.856$; Expt Ben 1, $p = 0.536$; Expt Ben2, $p = 0.856$).

Fig. 2. Effects of supplemented planktonic algae and light-stimulated growth of benthic algae on the abundance of heterotrophic flagellates (HFJs). (a,b) Mean ($\pm$ SD) abundance of HFJs in flow cells supplemented with suspended algae (Pla ENR) or a filtered (pore size 1.2 μ m) algae-free extract of the algal suspension (Pla CON) in Expts (a) Pla1 (carried out at a high temperature) and (b) Pla2 (carried out at a low temperature). (c,d) Mean ($\pm$ SD) abundance of HFJs in flow cells with increased light intensity (Ben LIGHT) or kept in darkness (Ben DARK) in Expts (c) Ben1 (moderate temperature) and (d) Ben2 (low temperature). See Fig. 1 and Table 1 for details on experiments and treatments

Effects of supplemented planktonic algae on ciliate biofilm development

Supplementation of planktonic algae (Pla ENR) consistently resulted in a highly significant stimulation of ciliates in both experiments (Fig. 3a,e, Table 2). However, the magnitude of responses clearly differed with respect to the dates of the experiments; while the total ciliate abundances in Pla CON and Pla ENR were already divergent after 7 d of succession (Fig. 3a) in Expt Pla1 (performed at higher water temperatures), ciliate abundances in Expt Pla2 (performed at lower water temperatures) were not different within the treatments until Day 10 (Fig. 3e). Furthermore, the maximum ciliate abundance was 9 times higher in Expt Pla1 on

Table 1. Overview of experiments, including time periods as well as water temperature and plankton abundance observed in the field water from the River Rhine. Plankton data are means $\pm$ SD. Temperature data are means of daily averages (range in parentheses). See Fig. 1 for details on treatments

Expt	Treatment	Time period (dd/mm/yy)	Temp. (°C)	Plankton abundance (cells ml ⁻¹)	
				Bacteria	Algae
Pla1	Pla ENR Pla CON	22/08/07 to 05/09/07	20 (18–21)	$1.6 \pm 0.2 \times 10^6$	420 ± 110
Ben1	Ben LIGHT Ben DARK	08/10/07 to 24/10/07	15 (12–17)	$1.1 \pm 0.1 \times 10^6$	180 ± 20
Pla2	Pla ENR Pla CON	14/11/07 to 10/12/07	8 (7–9)	$1.4 \pm 0.2 \times 10^6$	150 ± 20
Ben2	Ben LIGHT Ben DARK	14/11/07 to 10/12/07	8 (7–9)	$1.4 \pm 0.2 \times 10^6$	150 ± 20

Day 9 (60 ± 25 ind. cm^{-2} in Pla CON and 550 ± 190 ind. cm^{-2} in Pla ENR; Fig. 3a) and only 5 times higher in Expt Pla2 on Day 17 (140 ± 15 ind. cm^{-2} in Pla CON and 720 ± 140 in Pla ENR; Fig. 3e) in Pla ENR compared to Pla CON.

Regarding the taxonomic composition, the strongest effects were detected for Peritrichia, which were significantly stimulated by *Chlorella* sp. (Pla ENR) in both experiments (Table 2). In Expt Pla1, they made up 20% of the total ciliate abundance in Pla CON (Fig. 3b) and 71% in Pla ENR (Fig. 3c) on Day 12. In Expt Pla2, the proportion of Peritrichia was higher than in Expt Pla1 (65% in Pla CON and 80% in Pla ENR on Day 20; Fig. 3f,g). Opposite observations were made for Choreotrichia, the abundance of

Fig. 3. Results of (a–d) Expts Pla1 (August to September 2007) and (e–h) Pla2 (November to December 2007) on the effects of supplemented planktonic algae on the early development of biofilm-dwelling ciliate communities. (a,e) Development of the mean ($\pm$ SD) ciliate abundances in the treatment with a supplement of planktonic algae (Pla ENR) or a supplement of filtered ($1.2 \mu\text{m}$), algae-free algal suspension (Pla CON). (b,f) Taxonomic composition (as percent contribution of ciliate groups to total ciliate abundance) of the ciliate community in Pla CON. (c,g) Taxonomic composition of the ciliate community in Pla ENR. (d,h) Non-metric multidimensional scaling (NMDS) based on the Bray–Curtis similarity of all replicates per treatment. Significantly different clusters identified using a SIMPROF test are encircled. See Fig. 1, Table 1 for details on experiment and treatments

Table 2. Results of the repeated measures ANOVAs (rmANOVAs) calculated to test the effects of supplements of planktonic algae and of light-stimulated benthic algal growth on the development of the total ciliate abundance and on the abundance of ciliate taxonomic units. Only those units that showed significant effects from the resources, with subsequent Dunn–Sidak correction, are listed. No taxonomic group showed significant effects of the treatment in Expt Ben1. Only the results of between-subject factors (resources) are given. See Fig. 1, Table 1 for details on treatments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Expt	Treatment	Total abundance <i>F</i> ratio and df	Abundance of taxonomic ciliate groups Taxonomic ciliate units	<i>F</i> ratio and df
Pla1	Pla ENR Pla CON	$F_{1,4} = 35.95^{**}$	Peritrichia Hymenostomatia	$F_{1,4} = 206.8^{***}$ $F_{1,4} = 31.8^*$
Ben1	Ben LIGHT Ben DARK	$F_{1,6} = 41.60^{***}$	–	–
Pla2	Pla ENR Pla CON	$F_{1,5} = 319.72^{***}$	Choreotrichia Peritrichia	$F_{1,5} = 18.3^*$ $F_{1,5} = 56.5^{**}$
Ben2	Ben LIGHT Ben DARK	$F_{1,6} = 10.87^*$	Nassophorea Stichotrichia	$F_{1,6} = 17.5^*$ $F_{1,6} = 43.6^{**}$

which was reduced in Pla ENR (significant for Expt Pla2; Table 2); they contributed 45% of the ciliate communities in Pla CON and 5% in Pla ENR on Day 12 of Expt Pla1 (Fig. 3b,c). In Expt Pla2, they contributed a maximum of 40% in Pla CON and 20% in Pla ENR (Fig. 3f,g). The NMDS plots of the Bray–Curtis similarity, which were calculated for the last day of each

experiment (Day 12 for Expt Pla1 and Day 20 for Expt Pla2), consistently showed clear separation between the ciliate communities in the Pla CON and Pla ENR treatments (Fig. 3d,h). This separation was significant (SIMPROF test, $\alpha < 0.05$) for Expt Pla1. See Appendix 1 (Table A1) for ciliate morphotypes observed in the present study.

Fig. 4. Results of Expts (a–d) Ben1 (October 2007) and (e–h) Ben2 (November to December 2007) on the effects of light-stimulated benthic algae on early biofilm-dwelling ciliate communities. (a,e) Development of the mean ($\pm$ SD) ciliate abundance with elevated light exposure (Ben LIGHT) and the dark control (Ben DARK). (b,f) Taxonomic composition of the ciliate community in Ben DARK, given as contribution of ciliate groups to total ciliate abundance, and (c,g) in Ben LIGHT. (d,h) Non-metric multidimensional scaling (NMDS) based on the Bray–Curtis similarity of all replicates per treatment. Significantly different clusters identified using a SIMPROF test are encircled. See Fig. 1, Table 1 for details on experiments and treatments

Effects of light-stimulated benthic algal growth on ciliate biofilm development

Artificial illumination (Ben LIGHT) resulted in strong stimulation of surface-attached microalgae, especially the species of unicellular and filamentous diatoms. In Expt Ben1, the abundance of benthic algae in Ben DARK was rather low (final abundance: 2.0 ± 4.5 cells cm⁻²). In Ben LIGHT, the abundance of benthic algae increased exponentially during the experiment, reaching a maximum of 2750 ± 1490 cells cm⁻² on Day 14. In Expt Ben2, the final abundance of benthic algae was 1.0 ± 1.5 cells cm⁻² in Ben DARK and $52\,700 \pm 13\,050$ cells cm⁻² in Ben LIGHT on Day 24.

Illumination also resulted in significant effects on the total abundance of ciliates in Expt Ben1 (Fig. 4, Table 2) and on both total abundance and community structure in Expt Ben2 (Fig. 4, Table 2). In Expt Ben1, total abundances of ciliates in Ben LIGHT and Ben DARK were equal until Day 5 (Fig. 4a). In the following days, the abundance of ciliates in Ben DARK increased linearly from 45 ± 10 ind. cm⁻² on Day 5 to 70 ± 25 ind. cm⁻² on Day 14 (Fig. 4a), whereas in Ben LIGHT we observed an exponential increase to 215 ± 55 ind. cm⁻² (steady-state abundance) on Day 10.

In Expt Ben2, the total abundances of ciliates in Ben DARK and Ben LIGHT increased in parallel until Day 17 (Fig. 4e), when we recorded 160 ± 65 ind. cm⁻² in Ben DARK and 150 ± 40 ind. cm⁻² in Ben LIGHT. In the following days, the abundance of ciliates developed completely differently in the 2 treatments. At the end of the experiment (Day 27), the abundance of ciliates was 210 ± 75 ind. cm⁻² in Ben DARK and 610 ± 60 ind. cm⁻² in Ben LIGHT.

Regarding taxonomic composition, Ben LIGHT resulted in a selective increase in distinct taxonomic units (Fig. 4b,c,f,g);

while taxonomic composition did not differ significantly between Ben LIGHT and Ben DARK in Expt Ben1, single taxonomic units were stimulated in Expt Ben 2 (Table 2). Thus, the taxonomic responses observed in Expt Ben2 (performed at lower ambient water temperatures) were different in that Nassophorea and Stichotrichia responded significantly to the stimulation by benthic algae (Table 2, Fig. 4g). Nassophorea increased from Day 17 onwards and contributed 4 % of the total ciliate abundance in Ben DARK and 22 % in Ben LIGHT on Day 24. Stichotrichia, which also showed significant treatment effects, reached at maximum 0.5 % in Ben DARK and 25 % in Ben LIGHT. Peritrichia, which were dominant in the Ben DARK treatment towards the end of the experiment (75 and 70 % on Days 17 and 24, respectively), made up only 40 % (Day 17) and 13 % (Day 24) of the total ciliate abundance in the Ben LIGHT treatment. Because the absolute abundances of Peritrichia were similar in the 2 treatments of Expt Ben2, no significant treatment effect could be detected (Table 2). NMDS plots of the Bray–Curtis-similarity, which were calculated for the last days of the experiments, did not reveal significant treatment effects (SIMPROF test; $p > 0.05$) in Expt Ben1 (Fig. 4d). In Expt Ben2, 2 significant clusters (SIMPROF test; $\alpha < 0.05$) that separated the Ben LIGHT from the Ben DARK treatment were obtained (Fig. 4h). A list of ciliate morphotypes observed during the present study is given in Appendix 1 (Table A1).

DISCUSSION

To our knowledge, this is the first study to test the impact of directly increased densities of microalgae on natural ciliate biofilms (but see Hillebrand et al. 2002, Wickham et al. 2004 for fertilization experiments). The high temporal resolution of the community analyses that we performed was facilitated by the application of flow-cell systems: unlike conventional surveys, the flow cells permit documentation of processes that appear in short chronological sequences.

As hypothesized when planning the experiments, early biofilm colonization by ciliates was generally not affected by algal supplements due to the lack of algivorous species that could have been stimulated; conversely, later stages in the succession of ciliates in the biofilm (i.e. maturation) showed clear positive responses to increased availability of both planktonic and benthic algae. This contrasts with the observations made previously by Norf et al. (2009a) in experiments with supplements of planktonic and benthic bacteria in that both early colonization and maturation of ciliate biofilms was stimulated by the bacterial supplements. Taken together, these results confirm earlier assump-

tions on the functional succession of surface-dwelling ciliates (cf. Franco et al. 1998).

Remarkable differences in the responses to algal supplements were also observed between the seasonal experiments—probably due to differences in water temperature. It is well understood that several metabolic processes, such as feeding and growth rates, are controlled by temperature (Montagnes & Weisse 2000, Weisse et al. 2002). Norf & Weitere (2010) have shown that both temperature and the availability of planktonic bacteria are important factors controlling the development of ciliate biofilms. Our data confirm these results in that the ciliate communities responded much more slowly to increased food availability at lower, compared with higher, water temperatures.

Enrichment with planktonic algae affects ciliate abundance and biofilm structure

By far the strongest effects on ciliate abundance were detected in the experiments with supplements of planktonic algae. The stimulation of Peritrichia, in particular, demonstrates a strong direct benefit for these suspension-feeding ciliates (cf. Eisenmann et al. 2001, Kathol et al. 2009) from the enrichment with suspended algae. The increase in Peritrichia was accompanied by a reduction in suspension-feeding Choreotrichia (exclusively *Tintinnidium* sp. and *Strobilidium caudatum*) in both experiments because both groups occupy similar functional niches. Norf et al. (2009a) obtained similar results for enrichment with planktonic bacteria. A likely explanation is that Peritrichia, most of which have stalked cell bodies, could potentially graze upon particles in higher layers of the water column. This could result in a limitation of resources in the lower biofilm layers and, consequently, cause a decrease in other suspension-feeding ciliates such as Choreotrichia. Such a competitive advantage of Peritrichia, compared to other suspension-feeding ciliates, could explain the dominance of Peritrichia in most mature ciliate biofilms (Gong et al. 2005, Primc-Habdija et al. 2005, Norf et al. 2009b, Kathol et al. 2011).

In addition to the clear and highly significant effects of Pla ENR on suspension-feeding ciliates (see above), significant effects were also observed for surface-browsing groups such as Hymenostomatia. In our case, *Glaucoma scintillans* and *Lembadion lucens* showed positive responses to supplementation with planktonic algae. We suspect that the bacterivorous *G. scintillans* was most likely stimulated by enhanced growth of biofilm bacteria (cf. Huws et al. 2005, Weitere et al. 2005), whereas the positive response of the (mostly predatory) *L. lucens* could have resulted from an increase in smaller bacterivorous microbes such as

HFs and small ciliates. Especially the grazing of *L. lucens* upon HFs could explain why no significant effects of Pla ENR on HFs were detected. However, it is remarkable that stimulation of bacterivorous ciliates was observed only in the Pla ENR treatments, which presumably contained the same amount of DOM as the control treatments. We suspect that enhanced activity of suspension feeders and, consequently, increased release of metabolites (including DOM) near the biofilm surface could stimulate benthic bacterial production (cf. Zubkov & Sleigh 1999). It should be noted that even stalked Peritrichia can occur within the bacterial biofilm matrix, as has been recently shown with laser-scanning microscopy (Villanueva et al. 2011). In this way, even planktivorous Peritrichia could make DOM available for the biofilm food web.

Direct and indirect effects of benthic algae on surface-feeding ciliates

Towards the end of the experiments with artificial illumination (Ben LIGHT), we observed both a significant increase in ciliate abundance and an accumulation of filamentous algae. However, ciliates reached steady-state abundances after 12 to 15 d of biofilm development in Expt Ben1, while benthic microalgae continued increasing until the end of the experiment. It is likely that most algal cells within the maturing biofilms were resistant to grazing, e.g. by secretion of extracellular polymeric substances (EPS) or by the formation of grazing-resistant morphotypes (e.g. large cell chains in *Melosira* sp.). In fact, the majority of microalgae in the mature biofilms were filamentous. On this account, Barranguet et al. (2005) reported that the presence of grazers increases the density of grazing-resistant algae in biofilms. Yet another possible explanation is that the ciliates had reached carrying capacity due to space limitation. However, we do not favor this explanation because remarkably higher densities of ciliates were previously reported in similar experiments (Norf et al. 2007, 2009a).

Regarding ciliates, artificial illumination—and thus enhanced growth of benthic algae—results in an increase particularly in benthivorous taxa, as was expected when planning the experiments. In Expt Ben2, performed during the fall, Nassophorea and Stichotrichia, in particular, were positively affected; both of these groups usually occur during seasons of phytoplankton and phytobenthos blooms (spring and late summer). In Expt Ben2, the stimulation of Stichotrichia resulted in the formation of a ciliate community that is rather atypical for this time of the year (i.e. fall) in the River Rhine (cf. Norf et al. 2009b).

Besides the direct consumption of algae by surface-browsing ciliates (e.g. *Chilodontopsis depressa*, *Chilodonella uncinata*), selected ciliates could also benefit from stimulated benthic bacterial growth, e.g. due the release of algal exudates (Grossart & Simon 2007). Goedkoop et al. (1997) showed that supplementation with diatoms can stimulate both the activity and biomass of bacteria and subsequently increase the abundance of ciliates. In the present study, indications for such indirect effects of light-stimulated algal growth are rendered from the stimulation of bacterivorous ciliates such as *Hexotricha caudata* (Nassophorea) and *Cinetochilum margaritaceum* (Hymenostomatia). As mentioned above, we detected no effects of Ben LIGHT on HFs; this could be due to rapid trophic transfer of DOM via bacteria and HFs to ciliates. On this account, Norf et al. (2009a) reported that supplementation with dissolved organic carbon in developing biofilms can lead to (temporary, visible) stimulation of HFs that are immediately grazed by ciliates. Another likely explanation is that the maturing bacterial biofilms were resistant to grazing by HFs but were not resistant to grazing by ciliates (cf. Weitere et al. 2005).

Conclusion on the position of ciliates in the biofilm food web

There is full awareness of the significant role of ciliates in linking primary production to higher trophic levels within planktonic food webs (e.g. Azam et al. 1983). However, in biofilm food webs the role of protozoans is apparently underestimated. The current understanding of benthic food webs is that the primary production on surfaces is predominantly consumed by the grazing macrofauna (cf. Wetzel 2001). The present study shows that parts of the autotrophic primary production are utilized by the microfauna, as well. Consumption of planktonic and benthic algae by ciliates represents 2 different energy channels through which primary production enters the biofilm consumer food web.

Both of the experimental resource increments resulted in both significant increases in total ciliate abundance and significant community effects. Both variables can also have severe influences on the energy flow within microbial food webs. Further investigations are needed to help disentangle the quantitative importance of ciliates in benthic food webs.

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Appendix 1. Additional taxonomic data

Table A1. Ciliate morphotypes observed during experiments with supplementation of planktonic algae (Type Pla) and with light-stimulated growth of benthic algae (Type Ben). Pla1 and Pla2 refer to experiments carried out at high and low temperatures, respectively. Ben1 and Ben2 refer to experiments carried out at moderate and low temperatures, respectively. For further details, see Fig. 1 and Table 1

Rank	Morphotypes	Experiments and treatments							
		Pla1		Ben1		Pla2		Ben2	
		Pla CON	Pla ENR	Ben DARK	Ben LIGHT	Pla CON	Pla ENR	Ben DARK	Ben LIGHT
Choreotrichia	<i>Strobilidium caudatum</i>	x	x	x	x	x	x	x	x
	<i>Tintinnidium</i> sp.	x	x	x	x	x	x	x	x
	Undetermined		x					x	
Cyrtophoria	<i>Chilodonella uncinata</i>	x	x	x	x		x	x	
	<i>Chilodontopsis depressa</i>					x	x	x	
	<i>Dysteria fluviatilis</i>		x						
	<i>Gastronauta</i> sp.		x		x	x	x	x	x
	<i>Odontochlamys alpestris</i>		x						
	<i>Pseudochilodontopsis</i> sp.		x				x		
	<i>Thigmogaster oppositevacuolatus</i>	x	x						
	<i>Thigmogaster potamophilus</i>		x						
	<i>Thigmogaster</i> sp.	x	x			x	x	x	x
	<i>Trochilia minuta</i>						x		
	Undetermined	x	x	x	x	x	x	x	x
Heterotrichea	<i>Stentor</i> sp.	x	x		x				x
Hymenostomatia	<i>Glaucoma scintillans</i>	x	x			x	x		x
	<i>Lembadion lucens</i>	x	x	x		x	x	x	x
	Undetermined		x	x	x	x	x	x	x
Hypotrichia	<i>Aspidisca circada</i>	x	x	x	x	x	x	x	x
	<i>Aspidisca lynceus</i>	x	x	x		x	x	x	x
	<i>Aspidisca</i> sp.	x	x	x	x	x	x	x	x
	<i>Euplotes</i> sp.		x			x		x	
Litostomatea	<i>Acineria incurvata</i>	x	x					x	
	<i>Acineria</i> sp.		x				x	x	x
	<i>Acineria uncinata</i>	x	x						
	<i>Amphileptus procerus</i>	x							
	<i>Amphileptus</i> sp.							x	
	<i>Litonotus alpestris</i>	x	x			x	x	x	x
	<i>Litonotus crystallinus</i>		x			x			x
	<i>Litonotus cygnus</i>	x	x		x			x	
	<i>Litonotus lamella</i>	x	x			x	x	x	x
	<i>Litonotus</i> sp.							x	
	Undetermined	x	x	x	x	x	x	x	x
Nassophorea	<i>Chilodontopsis depressa</i>	x	x		x	x	x	x	x
	<i>Microthorax pusillus</i>		x						
	<i>Zosterodasys transversa</i>								x
	Undetermined	x	x	x	x	x	x	x	x

(continued on next page)

Table A1 (continued)

Rank	Morphotypes	Experiments and treatments							
		Pla1		Ben1		Pla2		Ben2	
		Pla CON	Pla ENR	Ben DARK	Ben LIGHT	Pla CON	Pla ENR	Ben DARK	Ben LIGHT
Peritrichia	<i>Campanella umbellaria</i>							x	x
	<i>Carchesium polypinum</i>		x	x		x	x	x	x
	<i>Epistylis</i> sp.				x	x		x	x
	<i>Vorticella aquadulcis</i> -complex	x	x						
	<i>Vorticella campanula</i>	x	x			x	x	x	x
	<i>Vorticella picta</i>	x	x	x	x	x	x	x	x
	<i>Vorticella</i> sp.	x	x	x	x	x	x	x	x
	<i>Zoothamnium procerius</i>		x			x	x	x	x
	Undetermined	x	x	x	x	x	x	x	x
Prostomatea	Undetermined		x						
Scuticociliatia	<i>Cinetochilum margaritaceum</i>	x	x	x	x	x	x	x	x
	<i>Cyclidium glaucoma</i>	x	x						
	<i>Uronema nigricans</i>	x							
	Undetermined	x	x	x	x	x	x	x	x
Stichotrichia	<i>Holosticha pullaster</i>	x	x	x	x	x	x	x	x
	<i>Holosticha</i> sp.	x	x						
	<i>Oxytricha</i> sp.	x	x						
	<i>Stylonychia mytilus</i>	x	x		x		x		x
	Undetermined	x	x	x	x	x	x	x	x
	<i>Uroleptus piscis</i>		x		x				x
Suctoria	Undetermined	x	x					x	

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