Control of biofilm-dwelling ciliate communities by temperature and resources

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Slàinte math!

Gälische Weisheit

Content:

General Introduction

Microbial biocoenoses play an important role in the matter flux of aquatic ecosystems (Finlay & Esteban 1998). The major part of the microbial activity is concentrated on surfaces, particularly in shallow and running waters, (Bryers 1982, Fischer & Pusch 2001), where the microbes are assembled in so-called biofilms (Wetzel 2000). Recent investigations have emphasised the functional role of ciliate communities in importing resources from the plankton into the benthos (cf. Weitere & Arndt 2003). Their productivity is suggested to equal or even exceed invertebrate production (Finlay & Esteban 1998). Biofilm-dwelling ciliate communities are composed of a variety of taxa which strongly differ in their ecological function. Many ciliates are suspension feeders, meaning that they can acquire their resources from the water flow. Other ciliates can collect food items (e.g. bacteria, microalgae, and heterotrophic flagellates) from the biofilms or can prey on larger organisms such as ciliates or micrometazoans. Besides the total organism density, the function of biofilm-dwelling ciliate communities is thus coupled to the species composition. The species composition itself is controlled by several factors that often interact. For this reason, the designation of community responses towards changes in particular environmental variables is often complicated.

Whereas planktonic ciliate community dynamics have been well studied over the last few decades, little is known about the control of biofilm-dwelling ciliate communities. Reason for this are the difficulty in accessing biofilms and the fact that many techniques known from plankton research (e.g. size fractioning, dilution experiments) cannot easily be adopted for microbial biofilms. One remedy was detected in bacterial biofilm research by the invention of miniature flow cells in which bacterial biofilms can be cultivated under controlled laboratory conditions (e.g. Stoodley et al. 1999). The flow cell principle then was adopted for field-related experiments aboard the Ecological Rhine Station (University of Cologne) - a former boat tender which offers the opportunity to conduct ecological experiments with naturally grown biofilm communities (Eßer 2006). The biofilms are cultivated in flow cells used as

bypass systems with a permanent flow of untreated river water. Thereby, the river water serves as both the species pool for the settlement of the organisms from the water flow (cf. Scherwass & Arndt 2005) as well as the resource reservoir for the nutrition and thus the maintenance of the biofilm communities.

Though functioning, the time-span of these experiments was often restricted to a couple of days due to the sedimentation of fine-grained particulate matter from the water flow and thus the disruption of the biofilm-dwelling microbial communities. The first aim of the present thesis thus was to refine the available flow cell systems in order to prolong the usefulness of the flow cells. A detailed description of the flow cells is given later in the thesis (Chapter 1). After testing, the flow cell systems were implemented in different experiments with different manipulations to test the impacts of mimicked environmental changes on the development and on the structure of biofilm-dwelling ciliate communities.

One of the most striking environmental changes is linked to recent global climate change and associated temperature increase (IPCC 2007) that is expected to constitute one of the major challenges for ecological communities. Although laboratory studies have shown that ciliates can respond to small changes in temperature (cf. Laybourn & Finlay 1976, Weisse et al. 2001, Jiang & Morin 2004), the possible responses of natural communities towards temperature increases remain largely unknown. Thus, the first part of this thesis investigated the impacts of enhanced temperatures on the development of complex, biofilm-dwelling ciliate communities. These experiments were part of a priority programme of the German Research Foundation (DFG) named AQUASHIFT, which investigates the impacts of temperature increase on ecological communities as a consequence of anthropogenic induced warming.

Chapter 1 concentrates on the impact of enhanced temperatures on the early development of ciliate communities which had been cultivated in flow cells starting from sterile surfaces. This work included investigations of possible seasonal dependencies of temperature responses, acknowledging that the environmental setting could influence the magnitude of responses towards warming. Whereas this work concentrated on the numerical development of

ciliate communities, the taxonomic responses of ciliate communities towards in particular summer- and winter warming are discussed in detail in **Chapter 2**. It was also tested in how far short-term community responses to temperature increases during winter could be forwarded to later stages in the development of biofilm-dwelling ciliate communities. In order to test the mechanisms of temperature responses, one additional summer experiment with cross-manipulations of temperature and resource supplements was performed.

Another important factor when addressing community responses towards environmental changes is the availability of resources. Since field studies have shown that ciliate communities can respond to increased resource levels, the results of these studies are partially controversial. Whereas e.g. Domenech et al. (2006) reported strong responses of ciliate communities towards resource enrichments, Wilcox et al. (2005) found no effects of similar resource enhancements on ciliates. These opposing results could be explained by different aspects: First, most studies concentrate on a few surveys within a relatively broad time-span, which increases the risk of short-term responses towards resource enrichments being missed. Secondly, resource enrichments in the field are mainly performed by stimulation of producers with nutrients (e.g. glucose, fertilizer) with indirect effects on the consumer community. Little is as yet known on how complex field communities could respond to direct resource enhancements (e.g. by the addition of bacteria).

Chapter 3 presents the results from experiments with benthic and planktonic resource manipulations using flow cells. In four experiments covering different seasons, a solution containing an additional carbon source was added to the water flow in the flow cells in order to enhance the growth of benthic bacteria; a suspension of planktonic bacteria was also added. Therefore it was possible to compare in how far the development of biofilm-dwelling ciliate communities can be influenced by enhanced resource densities from different origins. Acknowledging that the effects of resource enhancements could differ between early and late (mature) ciliate communities, it was further tested in how far late,

pre-cultivated biofilm-dwelling ciliate communities could respond to an enhanced density of planktonic bacteria. These results are presented in **Chapter 4**. Therefore, a novel type of flow cells was designed to facilitate manipulation and non-destructive observation of pre-cultured biofilm-dwelling ciliate communities to test the responses of mature ciliate communities towards the resource enhancement.

TEMPERATURE CONTROL OF CILIATE COMMUNITIES

Chapter 1.

Impact of local temperature increase on the early development of ciliate communities.

Abstract

Indications of global climate change and associated unusual temperature fluctuations have become increasingly obvious over the past few decades. Consequently, the relevance of temperature increases on ecological communities and on whole ecosystems is one of the major challenges of current ecological research. One approach to investigating the effects of increasing temperatures on communities is the use of fast-growing microbial communities. Here we introduce a river bypass system in which we tested the effect of temperature increases (0, 2, 4, 6°C above the long-term average) on both the colonization speed and the carrying capacity of biofilm-associated ciliate communities under different seasonal scenarios. We further investigated interactions of temperature and resource availability by cross manipulations in order to test the hypothesis that temperature-meditated effects will be strongest in environments which are not resource-limited. Strong seasonal differences in both tested parameters occurred under natural conditions (no resource addition), while the effects of temperature increase at a given time were relatively low. However, increasing temperature can significantly accelerate the colonization speed and reduce the carrying capacity in particular seasons. These effects were strongest in winter. Simultaneous manipulation of temperature and of resource availability amplified the response to temperature increase, adumbrating strong interactive control of populations by temperature and resource availability. Our results show that the response of communities to

local temperature increases strongly depends on the seasonal setting, the resource availability and the stage of succession (early colonization speed vs. carrying capacity).

Introduction

Over the past few decades, global surface and surface water temperature have been increasing as a consequence of anthropogenic green-house gas emissions (IPCC 2001). The impact of this climate change is a source of lively discussion in a number of scientific disciplines. Ecologists were researching approaches for measuring and predicting environmental responses to changing climate regimes long before the current scientific interest in "global warming" was sparked (Andrewartha and Birch 1954; Wieser 1973). Today it is widely accepted that current global climate change broadly affects ecosystems (Walther *et al.* 2002). However, these effects can differ strongly and can cumulate in reorganization of whole ecosystems (Brown *et al.* 1997; McGowan *et al.* 1998; Sala *et al.* 2000). It remains a challenge to identify the factors that determine the intensity of ecosystem modifications due to climatic changes.

Next to body size, temperature is the strongest factor influencing an individual's metabolic rate (Gillooly *et al.* 2001; Savage *et al.* 2004). As the most fundamental physiological parameter, the metabolic rate influences numerous biological processes such as growth and feeding rates and thus also influences the interaction strength between organisms (Sanford 1999; Vasseur and McCann 2005). Within the optimal temperature range, increasing temperature stimulates population growth rates, which are then constrained by the availability of resources (Montagnes and Weisse 2000; Weisse *et al.* 2002). Furthermore, the ingestion rate can be increased by rising temperature, but again can be limited according to the availability of food items (Boenigk *et al.* 2002). If both the growth and ingestion rates were limited by resource availability rather than by temperature, increasing temperature would not significantly alter growth and feeding-related interactions. Consequently, resource availability could be one important factor limiting the intensity of community reactions to increasing temperature (Pomeroy and Wiebe 2001; Staehr and Sand-Jensen 2006).

Considering the future importance of local temperature increases, surprisingly little is known about the effect of temperature on the carrying capacity of

populations and communities (Savage *et al.* 2004). The population model by Savage *et al.* (2004) predicts that with constant resource supply the carrying capacity will decrease with increasing temperature in order to balance the effect of increasing metabolic costs. However, this prediction is not completely supported by the findings of Vasseur and McCann (2005), who argue that a temperature-mediated decrease in the carrying capacity most probably results from a decrease in the resource availability itself due to temperature increase. Nevertheless, a possible negative effect of temperature increase on late stages of succession might contrast a positive effect of temperature increase on early stages of succession.

Dispersal is another important factor currently in the focus of research on alterations in local community structure (e.g. in the metacommunity concept as reviewed by Leibold *et al.* 2004; Holyoak *et al.* 2005) which might be influenced by changing temperature conditions, as well (Clark *et al* 2003; Holzapfel and Vinebrooke 2005; Pearson 2006). Hence, one needs to consider not only growth and feeding rates but also temperature-related dispersal patterns when addressing community responses to warming. The complexity of possible interactions, however, makes it virtually impossible to predict consequences of recent climate change in natural communities. From the results of laboratory experiments, Jiang and Morin (2004) pointed out that responses of competing species to temperature were not predictable from observations of single species' responses to temperature increase. Even more, nonlinear responses to small fluctuations in the environment already occur in simple communities (Becks *et al.* 2005). Such difficulties in predicting community responses point out the imperative for applying different approaches in assessing community responses to recent climate change. One useful tool is the experimental hypothesis testing in fast-growing model communities of unicellular organisms (e.g. Fox and Morin 2001; Jiang and Morin 2004; Jiang and Kulczycki 2004).

Here we present an open flow cell system used as a bypass to a natural water body that allows investigation of biofilms over long-term periods. This system is suitable for the establishment of semi-natural biofilm communities and permits

experimental manipulation of many factors (e.g. temperature, nutrient load). Unlike batch assays, flow cells always allow dispersal of organisms, which is particularly important in unidirectional flowing river systems. In this type of system, the physical limitations of settlement or active choice of habitat in response to local environmental conditions can strongly influence the community composition. Our investigations focussed on fast-growing biofilm-associated ciliate communities that can rapidly colonize different boundary layers (Franco *et al*. 1998; Arndt *et al*. 2003), here the substratum-water interface of a river. Laboratory experiments have demonstrated strong grazing effects of biofilm-associated ciliates on planktonic organisms (Weitere *et al*. 2003) and thus place biofilms in a key position in linking planktonic and benthic food webs. The flow cells were fed from the River Rhine, where ciliates are resource-limited over most of the year (Scherwass and Arndt 2005). As a primary hypothesis, it was tested whether or not community responses to warming are limited in natural scenarios and if the addition of organic resources could lead to stronger temperature-related changes in community abundances. Furthermore, it was hypothesized, that early and late stages of succession are differentially sensitive against temperature increase. In four experiments over a complete annual cycle, the flow cells were exposed to an averaged field temperature as determined from the particular season's ambient temperatures (in the following: T_0) and to temperature elevations of 2, 4 and 6°C above T_0. In two additional experiments with cross-manipulation of temperature (T_0, T_0+6°C) and an additional organic carbon source (yeast extract), the interactive effect of resource availability and temperature increase was tested.

## Material and Methods

Study site and facilities

All experiments were performed aboard the Ecological Rhine Station, Cologne (Rhine km 684.5). The station is a former boat tender featuring several laboratories equipped with pump systems to allow a permanent supply of fresh

river water for experiments. As a tool for non-destructive observation of developing biofilms, miniature flow cells (Fig. 1a) were adapted from laboratory systems, where they are frequently used for experiments with bacterial biofilms (e.g. Stoodley and Warwood 2003). All flow cells were sealed with sterile microscopic slides at the bottom and cover slips at the top to guarantee optical quality with particular regards to video microscopy. The resulting internal space of the flow cells had a total surface area of 7.2 cm^2 and a total volume of 3.8 ml. Although this method also allows taxonomical classification of ciliate communities, this paper will exclusively focus on abundance estimates.

In the cultivation of biofilms using flow cells with river bypasses, some phenomena may affect long-term observation of protozoan communities. The development of destructive air bubbles, also known from laboratory experiments, can generally be eliminated by inserting a bubble trap (Fig. 1b) in front of the flow cell inflows. Another restricting factor appearing exclusively in experiments with river bypasses arises from the omnipresence of particle load, which can never be completely excluded from the water flow. Therefore, the bubble traps were broadened in function by installing the water inflow (from impeller pumps) in the lower part and the outflow (to miniature flow cells) in the upper part of the bubble traps, thereby creating a sedimentation zone for fine-grained particulate matter.
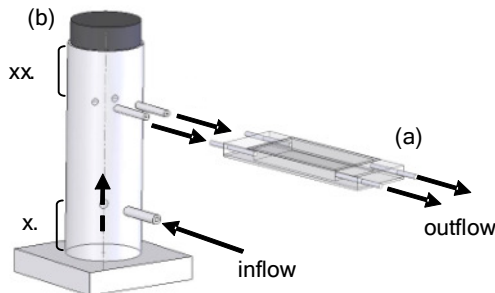
Fig. 1. Miniature flow cell system used for cultivation and *in situ* monitoring of semi-natural biofilms with permanent flow through of fresh river water. a: Miniature flow cell. b: Combined sediment (x) and bubble trap (xx).

All setups were permanently supplied with fresh and prefiltered (300 μm mesh size) river water using Watson Marlow® impeller pumps with an output of 2.5 ml min^{-1}. Chlorophyll a was regularly measured. Planktonic bacterial abundances (DAPI-counts) for each experiment (except July 2005) were kindly provided from routine observations by C. Viergutz and J. Dahlmann (University of Cologne). Information on DOC amounts were kindly provided by the Gas und Elektrizitätswerke Köln (GEW, Cologne, Germany).

Experimental set-up

Two types of experiments were performed. In the first type untreated river water was used in which the impact of gradual temperature increases on the development of early biofilm communities was investigated. In the second type the interactive effect of temperature and resource availability was investigated by manipulating both factors. Each experiment contained four different treatment regimes with three replicates each.

Table 1. Basic conditions during experiments. The ambient temperatures (T_0) for each experiment represent the semi-monthly average temperatures in the Rhine since 1989. The values for temperature manipulations refer to the corresponding T_0 for each experiment. The concentrations of dissolved organic carbon (DOC) and chlorophyll a (Chl) as well as the abundances of the planktonic bacteria represent the mean values over the period of the experiments.

Date	T_0 [°C]	Manipulations	DOC [mg l^{-1}]	Chl [μg l^{-1}]	Bacteria [ind. ml^{-1}]
2005					
March	10	+0, +2, +4, +6°C	2.54	7.5	6x10^5
May	19	+0, +2, +4, +6°C	2.17	34.5	9x10^5
July	23	+0, +6°C; add. resource	2.23	31.5	-
August	23	+0, +2, +4, +6°C	2.14	20.5	3x10^6
November	11.5	+0, +6°C; add. resource	2.37	10.5	9x10^5
2006					
January	6.5	+0, +2, +4, +6°C	2.75	25.5	9x10^5

The impact of local temperature increase on the early development of benthic ciliate communities was examined in March, May and August 2005 and in January 2006. For this purpose a setup with four consecutive temperature manipulations was chosen. The ambient temperature T_0, which was applied as reference value for all manipulations, was calculated from the semi-monthly averages (e.g. 1st half of January, 2nd half of January) recorded since 1989 in order to achieve likely representative temperature regimes for the seasonal experiments. Long-term temperature data were kindly provided by the Federal Institute of Hydrology (BFG, Koblenz, Germany). The flow cells including bubble traps were kept in temperature-controlled ($\pm0.5°C$) water baths. The temperature regimes were T_0 and manipulations of 2, 4 and $6°C$ above T_0 (see Table 1). In July and November 2005, two separate experiments were conducted to test for interactive effects of temperature (T_0, $T_0+6°C$) and resource availability. The resource level was manipulated by adding sterilized yeast extract suspension as an additional organic carbon source at a final concentration of 0.01 mg l^{-1} to enhance growth of benthic bacteria. In preliminary studies with different resource manipulations (0.001-0.1 mg l^{-1} yeast extract), this concentration was found to sufficiently induce nutrient-mediated effects on ciliate abundances and also avoided quick (<5 days) disruption of the experiments due to strong bacterial production.

The development of biofilm-associated ciliate communities was initially tracked daily, and then in two-day intervals starting on day five with a Zeiss Axioskop binocular microscope (50-630x magnification, phase contrast, camera tube). Ciliate abundances were repeatedly recorded in defined areas (0.016 cm^2) which were randomly distributed over the total cover slip area. The time-frame of these experiments ranged between two and four weeks. The experiments were stopped when no further significant increase in ciliate abundance was recorded. The data was then used to calculate the initial colonization speed as well as the carrying capacity of biofilms for ciliates. The finding of an early plateau of ciliate abundances was in accordance with earlier studies which found biofilm colonization to be characterized by the presence of an early

plateau that describes the time needed for initial colonization of biofilms by species (e.g. Pratt el al. 1986; McCormick *et al*. 1988; Hunt and Parry 1998). Although maturation of other microbial communities may take several weeks or even months to reach equilibrium (e.g. Cadotte *et al*. 2005), the early plateau was chosen as the basis for the calculations (in accordance with the specific biofilm studies mentioned above) in order to avoid a combination of effects caused by temperature manipulation and seasonal effects. For the treatment with resource addition (July and November 2005), data was only gathered for five days due to strong bacterial growth.

Data analysis

In order to quantify the response to temperature changes, both the carrying capacity and the colonization speed of succession were analyzed. Regression models were utilized to calculate the duration until 50 ind. cm^{-2} were present (t_{50}, days) and the carrying capacity (A_{max}; ind. cm^{-2}). The numerical increase of biofilm-dwelling ciliates in the early phase (approximately 1-5 days of succession) fits best to power function following the formula $A = a\, t^{\,b}$ with *A* being the abundance (ind. cm^{-2}) and *t* being the respective time (in days, d). The non-linear regression models were calculated using the SPSS® 11.0 software. These regression estimates were then used to determine the time span *t* needed until 50 biofilm dwelling ciliates were present (t_{50}) by solving the equations with a hypothetical ciliate abundance of $A = 50$ ind. cm^{-2}. The development of ciliate abundances over the total experimental phase was shown to follow a logistic curve given by the equation $A = a\,/\,(1+e^{b-ct})$. The parameters *a*, *b* and *c* were estimated with the help of curve fittings using SPSS®. This procedure directly provided the carrying capacity (A_{max}, ind. cm^{-2}) as represented by the parameter *a*. All curve fittings were performed separately for each temperature and replicate in the regular treatments (semi-natural conditions without addition of carbon source). After estimating t_{50} and A_{max} we performed one-factorial ANOVAs with temperature as predictor and t_{50} and A_{max} as dependent variables in order to test for significant effects of temperature

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increases on the particular parameter. The REGW-test was performed as post-hoc test for the multiple comparisons. In order to assess general effects of warming over all experiments, two-factorial ANOVAs were performed with the two temperature extremes (T_0, $T_0+6°C$) and date of experiment used as predictors and t_{50} and A_{max} as dependent variables. Application of these mathematical models was not possible in the treatments with resource addition as the experiments could not be run until A_{max} was reached. Instead, the abundances at day five (A_5, ind. cm^{-2}) were used as estimates for the colonization speed of early biofilms by ciliates. Here, statistical analyses were performed in a three-factorial ANOVA design with temperature, nutrient addition and date of experiment as independent variables.

Results

Temperature impact on the colonization speed under semi-natural conditions

In the first step we focused on the early colonization of biofilms by ciliates in order to check for possible effects of temperature increases on the colonization speed. The ciliate abundances within the early phase showed a good fit to power function (for r² values see Table 2), which allowed calculation of individual time spans until 50 ciliates were present on the biofilms (t_{50}). This value t_{50} exhibited a significantly high seasonal variability (two-factorial ANOVA: $p<0.001$, Table 3), with the most rapid colonization (lowest t_{50}) in August 2005 (2.1±1.1 days) and the slowest colonization (highest t_{50}) in November 2005 (8.0±1.7 days) for T_0 (Fig. 2A).

Compared to the large seasonal differences in t_{50}, the temperature effects within the experiments were low. No significant effect of temperature could be demonstrated between the temperature extremes of all experiments (T_0, $T_0+6°C$) (Table 3), despite a tendency towards a stimulation of the colonization speed with increasing temperature (Fig. 2B). Only in January, representing the experiment with lowest T_0 (8°C), did the experimental temperature increase account for a significant decline of t_{50} (one-factorial ANOVA: $p<0.001$, Table 4)

from 3.6±0.2 d for T_0 to 2.7±0.3 d for T_0+6°C. This reduction, however, did not occur stepwise. In fact both the "low" (T_0, T_0+2°C) and "high" (T_0+4°C, T_0+6°C) temperature regimes clustered with rather similar values for t_{50}.
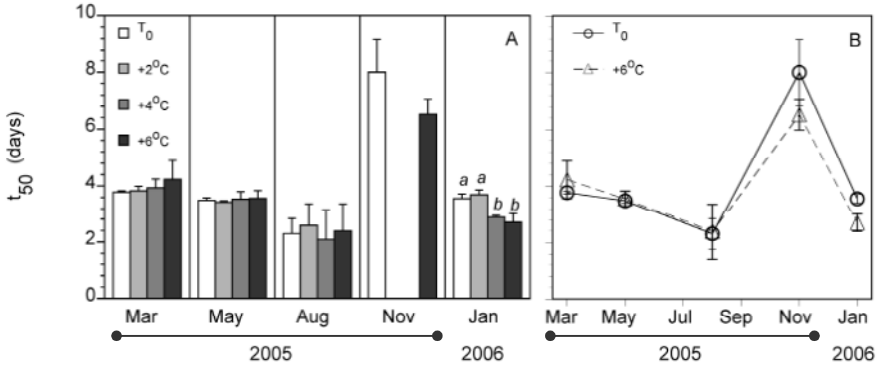
Fig. 2. Initial colonization speed (t_{50}) of semi-natural biofilms by ciliated protozoa. The value t_{50} represents the time span until an abundance of 50 ciliates cm^{-2} was reached on the biofilms. (A) Integrative diagram for all experiments and temperature treatments. (B) Values from temperature extremes (T_0, T_0+6°C) on real-time axis. Italics indicate significant differences between the treatments.

Table 2. R^2 values for non-linear regressions (power function) used to calculate the colonization speed (t_{50}) of biofilms by ciliates.

Temperature	T_0			+2°C			+4°C			+6°C		
Replicate.	1	2	3	1	2	3	1	2	3	1	2	3
2005												
March	.99	.99	.91	.98	.97	.99	.98	.98	.99	.99	.98	.91
May	.89	.96	.84	.88	.94	.95	.81	.83	.55	.96	.94	.83
August	.31	.91	.79	.73	.74	.99	.90	.85	.80	.25	.87	.48
November	.98	.98	.95	-	-	-	-	-	-	.99	.96	.98
2006												
January	.98	.97	.95	.97	.99	.93	.94	.96	.98	.98	.97	.96

Temperature impact on the carrying capacity (A$_{max}$) under semi-natural conditions.

Similar to the effects on t$_{50}$, the carrying capacities (A$_{max}$, determined from logistic regressions) were highly variable with the seasons (Fig. 3). A$_{max}$ was rather similar in March (230±50 ind. cm^{-2}) and November (190±25 ind. cm^{-2}). In May 2005, A$_{max}$ was notably higher with 320±35 ind. cm^{-2}. The experiment performed in August 2005 at highest T$_0$ (23°C) exhibited lowest carrying capacity with 110±20 ind. cm^{-2}. Ciliate abundances peaked in January with highest values for A$_{max}$ of 760±50 ind. cm^{-2}. All corresponding r² values for the logistic regressions may be extracted from Table 5. Temperature manipulation often led to a slight decrease in A$_{max}$, an effect which was strongest in the experiments with lowest T$_0$ (March 2005, January 2006) (Fig. 3A). Considering all experiments together, this reduction in A$_{max}$ with increasing temperature was significant for the temperature extremes (T$_0$, T$_0$+6°C; Table 3). However, when focussing on the individual experiments, a significant impact of temperature was recorded in August only (one-factorial ANOVA, p<0.001; Table 6).
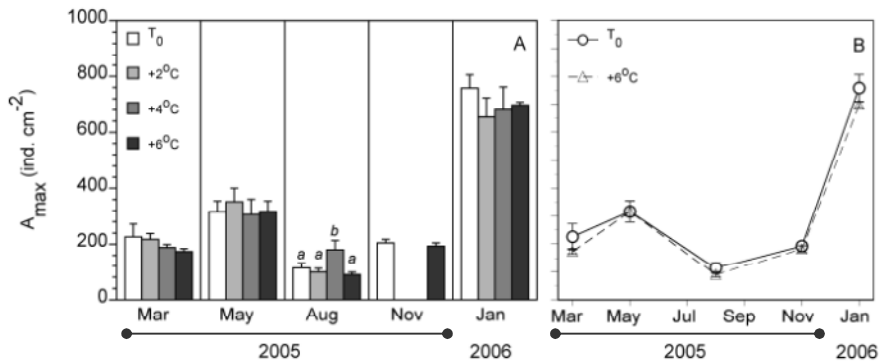
Fig. 3. Carrying capacity (A$_{max}$) of semi-natural biofilms for ciliates calculated and extracted from logistic regressions. (A) Integrative diagram for all experiments and temperature treatments. (B) Values from temperature extremes (T$_0$, T$_0$+6°C) on real-time axis. Italics indicate significant differences between treatments.

Table 3. Two-factorial ANOVA design for testing the effects of the experimental temperature extremes (T_0, $T_0+6°C$) on t_{50} and A_{max}. **Bold values** indicate significance.

Source	Square sum	df	F	p
t_{50}				
Temperature	0.902	1	2,549	0.126
Date of experiments	86,090	4	60,817	**<0.001**
Temperature x date	3,855	4	2,723	0.059
A_{max}				
Temperature	1,465,161	1	466	**<0.001**
Date of experiments	5,564	4	7.082	**0.015**
Temperature x date	4,806	4	1.533	0.232

Table 4. One-factorial ANOVA design for testing the effects of temperature on t_{50}. **Bold values** indicate significance.

Date		Square sum	df	F	p
2005					
March	Temperature	0.378	3	0.889	0.487
	Within treatments	1.132	8		
May	Temperature	0.030	3	0.262	0.851
	Within treatments	0.304	8		
August	Temperature	0.372	3	0.166	0.916
	Within treatments	5.968	8		
November	Temperature	3.286	1	3.958	0.117
	Within treatments	3.321	4		
2006					
January	Temperature	2.214	3	19.760	**<0.001**
	Within treatments	0.299	8		

Table 5. R^2 values for logistic regressions used to calculate the carrying capacity (A_{max}) of biofilms for ciliates.

Temperature	T_0			+2°C			+4°C			+6°C		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3
2005												
March	.99	.99	.91	.98	.97	.99	.98	.98	.99	.99	.98	.91
May	.89	.96	.84	.88	.94	.95	.81	.83	.55	.96	.94	.83
August	.31	.91	.79	.73	.74	.99	.90	.85	.80	.25	.87	.48
November	.98	.98	.95	-	-	-	-	-	-	.99	.96	.98
2006												
January	.98	.97	.95	.97	.99	.93	.94	.96	.98	.98	.97	.96

Table 6. One-factorial ANOVA design for testing the effects of temperature on A_{max}. **Bold values** indicate significance.

Date		Square sum	df	F	p
2005					
March	Temperature	5,401	3	2.3450	0.149
	Within treatments	6,142	8		
May	Temperature	6,521	3	1.2867	0.343
	Within treatments	13,515	8		
August	Temperature	15,237	3	11.4743	**<0.001**
	Within treatments	3,541	8		
November	Temperature	1	1	0.0000	0.996
	Within treatments	1,522	4		
2006					
January	Temperature	17,270	3	1.6763	0.248
	Within treatments	27,473	8		

Interactive effect of temperature and resource availability on initial colonization

We tested for interactive effects of temperature and resources on the initial speed of biofilm colonization by ciliated protozoa in two independent experiments. These experiments had to be terminated before equilibrating (because of strong bacterial reproduction within the flow cells with resource addition) and before 50 ciliates cm^{-2} were present on the biofilms in most treatments without resource addition. For this reason, we were not able to use the regression methodology (outlined in the methods section) to calculate the colonization speed, t_{50}. Instead, we used the abundance at day five (A_5) as a measure for the initial colonization speed.
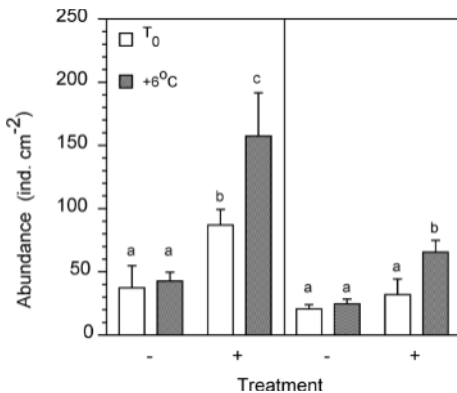
Fig. 4. Abundance of biofilm-associated ciliates after five days of succession in experiments with temperature (T_0, $T_0+6°C$) and resource (yeast extract) manipulation performed in July and November 2005. (-) No resource added. (+) Resource added. *Italics* indicate significant differences between treatments.

Table 7. Resource addition experiments: Result of a three-factorial ANOVA design for testing the effects of temperature T0, T0+6°C), resource quantity and date of the experiment (July and November 2005) on the ciliate abundance after five days of succession. **Bold values** indicate significance.

Source	SS	df	F	p
Date of experiments	12,395	1	24.5	**<0.001**
Resource addition	17,605	1	34.7	**<0.001**
Temperature	4,789	1	9.4	**0.006**
Resource x temperature	3,366	1	6.6	**0.018**

In both experiments (July and November 2005), the colonization-enhancing effect of temperature increase alone (no resource added) was low, consistent with the overall low temperature effect on t_{50} as mentioned above. However, with enhanced resources, an overall increase in ciliate abundance at day five was recorded with distinct differences between the two temperature treatments. This was supported by significant resource and temperature effects and by significant interactions between resources and temperature (Table 7, Fig. 4).

Discussion

The impact of increasing temperature on early biofilm-associated ciliate communities with and without resource addition was, for the first time, tested in "open" river bypass flow cell systems. Our results revealed strong seasonal variation in the time needed for initial biofilm colonization (t_{50}) and in the carrying capacity of biofilms for ciliates (A_{max}). Though temperature-mediated impacts on these colonization parameters were small in a seasonal context, they could be distinct and significant within single experiments. In particular, temperature increase can abbreviate the initial colonization time and reduce the carrying capacity. However, the only significant impact of increased temperature on t_{50} was found to occur during the colder seasons. Statistical comparison of the temperature extremes (T_0, $T_0+6°C$) did not reveal a significant overall effect of warming on t_{50} but did expose a significant impact of temperature on A_{max}. Furthermore, the largest effects of warming on the initial phase in biofilm succession were observed when additional nutrients were added, indicating that a strong interactive control by temperature and nutrients is a key factor in biofilm community development.

Temperature increase can reduce A_{max} at constant resource supply

When all experiments are considered together, a significant decline in A_{max} can be seen for a temperature increase of 6°C. These observations supported predictions derived from modellings done by Savage *et al.* (2004),

who concluded that when the resource supply is constant, the carrying capacity must decrease with increasing temperature to balance the effect of increasing metabolic costs. Additionally the magnitude of decline in A_{max} showed seasonal differences. Given that the resource levels themselves are seasonally different (Table 1), this suggests that both temperature and resource availability are crucial in determining A_{max}.

It should be noted that a comparison of the temperature extremes may be interpreted as two separate mechanisms: Temperature and possibly nutrient control at lower temperatures and a possible thermal intolerance of taxa at high temperatures might be the absolute limiting factors. Further taxonomic resolution of our data is necessary to obtain additional information about response differences between taxa, particularly with regards to the thermal tolerance of species. Stauffer and Arndt (2005) have shown that free-living freshwater protozoa may become extinct after a successive temperature increase within a narrow temperature range of between 28-30°C. Such extinctions of single taxa could result in modified interaction strength of the remaining species, which could then result in an unpredictable impact of temperature on communities. In this context, Jiang and Morin (2004) demonstrated that when species interact, a temperature increase can generate community changes which were not predictable from single species' responses. This could explain the results of the August experiment, which had the highest experimental temperature range (23-29°C) and incorporated both a significant increase of A_{max} (Fig. 3A) at a temperature elevation of 4°C (and a subsequent strong decrease of A_{max} between elevations of 4°C and 6°C) as well as noticeably high variability in t_{50} at all temperatures. However, a preliminary taxonomic assessment using families revealed no obvious temperature impact on biofilm compositions regarding ciliates at any season.

Temperature-mediated effects on the colonization speed depend on season and nutrient load

Experimental resource increases enhanced bacterial production, which likely resulted in an increase of predominantly picophagous ciliates which are typical pioneers in ciliate biofilm colonization (Franco *et al.* 1998; Arndt *et al.* 2003). Furthermore, the effect of the temperature increase was strongest after resource enhancement, giving rise to the conclusion that resource availability rather than temperature limited the colonization speed during the different seasonal conditions. The finding that stronger temperature-mediated effects only occurred when resources were not limited might explain the results recorded in January 2006. At this time, the strongest response to temperature increase occurred when the highest natural DOC load was found as well (Table 1). Thus, the seasonal differences in the response strength of t_{50} to temperature increases might be a result of the seasonally varying resource limitations of ciliates in the Rhine (Scherwass and Arndt 2005).

Recent studies have provided important information concerning the interactive control of ciliate growth by temperature and food supply. Weisse *et al.* (2002) have shown that even small adjustments of temperature and food supply can interactively alter the growth rates of ciliates in laboratory cultures. Furthermore, they showed that both factors can reach saturation levels. An interactive effect of temperature and resource quantity has also been found for other microbial communities such as bacteria and algae (Pomeroy and Wiebe 2001; Staehr and Sand-Jensen 2006). Taken together, these studies and our results suggest that different microbial communities can be buffered against climate-mediated temperature increases when resources are limited (see also Fox and Morin 2001).

Conclusion

Our results demonstrate that increasing temperature can result in different effects on the community density depending on the stage of succession, i.e. in an enhancement of the early colonization speed or in a reduction of the carrying capacity. Warming might thus result in contrasting effects on communities, depending on whether they are maintained in an early succession stage (e.g. in riverine biofilm communities which can undergo permanent disturbance due to sediment rafting) or in a late succession stage.

The magnitude of temperature-mediated effects, however, depends strongly on the environmental and seasonal conditions. Interestingly, the effects of temperature increases were found to be strongest in winter, which is the season for which the largest temperature increases forced by global warming are prognosticated (IPCC 2001). One important predictor for the magnitude of temperature-mediated effects is the availability of resources. Temperature impacts on aquatic communities can be expected to be strongest in resource-enriched systems such as eutrophic lakes (Felip *et al.* 1996; Bradshaw and Anderson 2001; Vrede 2005) or rivers and streams that carry high levels of organic material.

Chapter 2.

Structural responses of ciliate communities to local temperature increases.

Abstract

The impact of local temperature increase on the structure of consumer communities was experimentally tested. Fast-growing, biofilm-dwelling ciliates were used as model. In a first step we performed two seasonal experiments in which the ciliates were cultured for ten days in miniature flow cells at different temperatures.

Opposing effects of temperature increase appeared for "summer" and "winter" communities. In winter, the ciliates strongly benefited from enhanced temperatures in terms of both increased abundance and biomass resulting in significantly altered ciliate community compositions. Contrasting results were obtained in summer, when temperature increase resulted in a significant decline in ciliate biomass. At the same time, there was no significant temperature impact on the relative community composition. Based on these findings, we demonstrated in a further experiment that the results for winter are reproducible in mature, eight-week-old biofilms, i.e. that the carrying capacity increases with temperature in association with significant shifts in the community composition. The positive warming effects on the carrying capacity stands in contrast to expectations rooted in metabolic theory. By simultaneous manipulation of temperature and resource density in summer, it was further demonstrated that the negative warming effects on the carrying capacity could be compensated by increasing the availability of food, suggesting that energetic constraints rather than thermal limits for certain species are the main reason for

the observed effects. Furthermore, increased resource density resulted in significant temperature effects on the community composition.

Taken together, these findings show that the magnitude of responses to environmental warming in respect to both quantity and relative community composition strongly depends on the environmental setting, particularly the resource availability. The total community abundances can react differently towards warming than expected from population responses due to altered interaction strengths and associated community shifts.

Introduction

Ongoing global climate change and the associated environmental warming are considered to be one of the major ecological threats (Petchey *et al.* 1999, Hooper *et al.* 2005). Understanding the impacts of temperature increase on communities is thus an important challenge for ecologists. On an individual level, temperature is among the most important factors altering the metabolic rate and subsequently the survival, feeding and growth rates of most species (Gillooly *et al.* 2001). Within the species-specific optimal temperature range, increasing temperatures can enhance population growth rates (Savage *et al.* 2004; Alver *et al.* 2006) as long as the growth is not limited by resources (Felip *et al.* 1996; Weisse *et al.* 2002; Staehr and Sand-Jensen 2006). While responses to increasing temperatures have been well studied for many single species, there is still little understanding on how environmental warming could affect the structure of complex communities. Besides having an impact on individual metabolic rates, temperature can affect the interaction strength between organisms (Sanford 1999). Even relatively simple communities often have complex inter-specific interactions; environmental warming may thus have unpredictable consequences (Davis *et al.* 1998; Jiang and Morin 2004).

When the optimal temperature range is exceeded, species can experience thermal limitation which leads to range shifts (Portner 2002) and local extinctions (Thomas *et al.* 2004). The removal of a species from a community can in turn result in a decline in species diversity (Lloret *et al.* 2004; Burgmer *et al.* 2007), which could influence ecosystem function (Harley *et al.* 2006). Besides such warming-induced shifts in species composition due to different thermal optima of species, warming could also influence community density: One hypothesis states that the carrying capacity of a given community will decrease with increasing temperature in order to balance out the increased metabolic costs (at a constant resource supply) (Savage *et al.* 2004). This hypothesis is supported by findings from studies on trees and terrestrial ecotherms in which increasing temperature was found to reduce the abundance and the biomass of both plants and animals (Allen *et al.* 2002). In contrast,

Newsham and Garstecki (2007) found strong increases in the densities of heterotrophic flagellate communities when the temperature was experimentally increased. Similar results were obtained from observations of isolated populations of mountain lizards (Chamaille-Jammes *et al.* 2006). In contrast, a world-wide test on the effect of temperature on the density of lizard populations rejected any strong influence of environmental temperature on lizard population density (Buckley *et al.* 2008). Reasons can be behavioural thermoregulations and thermal adaptations.

The complexity and postulated unpredictability of community responses towards warming (Jiang and Morin 2004) imposes a challenge for ecologists trying to establish reasonable systems for testing the effects of warming on complex communities in addition to modelling approaches and to analyses of large-scale density patterns. An increasing number of studies in community and evolutionary ecology use fast-growing microbes to test ecological principles (Jessup *et al.* 2004; Weisse 2006). However, the trophic level of the microbes must always be considered, as different responses can occur for producer and consumer communities as shown in mesocosm experiments with experimental warming (Aberle *et al.* 2007; Sommer *et al.* 2007). Here we used complex, biofilm-dwelling consumer communities (composed of fast-growing ciliates) to test warming effects. Open bypass systems (miniature flow cells and flumes) were fed by a constant flow of untreated river water, thus allowing ciliate communities to establish and maintain themselves autonomously. In a previous study (Norf *et al.* 2007), we identified two patterns on how warming can alter community densities which were coupled to seasonal dependencies. In winter, increasing temperature significantly increased the colonisation rate of biofilms by ciliates, a result not found in summer. Furthermore, temperature increase reduced the carrying capacity of biofilms for ciliates. This phenomenon, however, was observed as a sum effect over several experiments and we were not able to attribute the effects on the carrying capacity to particular seasons. These previous findings necessitated further research for a better under-

standing of warming effects on mature consumer communities and of the mechanisms underlying these responses. We addressed the following questions in this study: (1) Do the effects of warming on the structure of mature consumer communities differ between summer and winter communities? (2) Are the warming effects on the community density associated to shifts in the community composition (expected when species react differentially towards warming due to specific thermal limitations) or do they affect each group symmetrically (as expected based on theoretical models that predict an increase in metabolic demands at constant resource supply)? (3) Are negative effects on the community density in the natural setting a consequence of limited resources, i.e. could they be compensated by supplemental resources?

Material and Methods

The experiments were performed aboard the Cologne Ecological Rhine Station of the University of Cologne (Cologne, Germany, Rhine-km 684.5, which refers to the from Lake Constance, the source of the non-alpine Rhine), a former boat tender that is equipped with laboratory facilities and pump systems to allow a permanent supply of fresh river water for ecological experiments. We used original Rhine River water containing both the planktonic organisms for the biofilm-colonization and the resources for the biofilm-dwelling consumers. Four experiments were performed. The first two experiments concentrated on the short-term effects of experimental warming on early ciliate communities during winter and during summer. Based on the results of these experiments, two other experiments were performed: In one long-term winter experiment, we tested whether or not the results from the short-term winter experiment were reproducible for mature biofilm-dwelling ciliate communities running at carrying capacity. In one additional summer experiment, we tested whether or not the observed negative warming effects could be compensated by supplemental resources.

Conduction of the warming experiments in river bypass systems

We conducted different experiments with manipulative local temperature increases in order to test the impact of simulated warming on early (ten days) and mature (eight weeks) biofilm-dwelling ciliate communities (hereafter: *early* and *late* ciliate communities). A summary of the experiments including the time frames and selected environmental variables in the River Rhine is given in Table 1. The two short-term experiments (10 days each) focused on the impact of a gradual temperature increase on early ciliate communities. The base temperature for the experiments was the calculated long-term average water temperature in the River Rhine since 1980 (hereafter: T_0). The primary tools for the investigations were miniature flow cells, which consisted of a clear acrylic frame with an object slide on the bottom and a cover slip on the top, enclosing an inner space of 3.6 ml volume. The flow cells were attached to a permanent bypass of untreated Rhine River water (flow rate: 3.8 ml m^{-1}) via tube pumps. A more detailed description of the flow cells is given in Norf *et al.* (2007). The flow cells were run in temperature-controlled water baths with particular temperature manipulations starting at T_0.

Table 1. Overview of the periods and conditions during the four experiments. The flow cell experiments were performed at constant temperatures (based on the long-term mean temperature at the particular season), whereas the flume experiment contained direct enhancement of the actual Rhine River temperature. Bacterial abundances were determined weekly by DAPI counts.

Experimental tools	Date	T_0	Treatments	Bacteria (10^6 ind. ml^{-1})
Flow cells (10 days)	Jan 19. - 29. 2006	6°C	T_0, +2, +4, +6°C	0.90±0.11
Flow cells (10 days)	July 25. - Aug 3. 2006	23°C	T_0, +3, +6, +9°C	1.21±0.22
Flow cells (10 days)	Aug 25. - Sep 3. 2007	24°C	Temperature x resource*	1.51±0.17
Flumes (8 weeks)	Nov 23. 2007 - Jan 15. 2008	6.1- 10.2°C	T_0, +3°C	1.09±0.10 - 1.36±0.15

* The temperature categories T_0 and +3°C were crossed with resource manipulations (ambient and supplemented bacteria)

Each treatment was run at least in triplicate. In January 2006 (hereafter: winter 2006), the applied temperature treatments were T_0 (6^oC for this season) and temperature increases of +2, +4 and $+6^oC$ above T_0. Data on the development of the ciliate quantity in this experiment were already presented in Norf *et al.* (2007) with regards to the colonization speed of the biofilms by ciliates and to the carrying capacity of the biofilms for ciliates. The counter-experiment was performed during August 2006 (hereafter: summer 2006). Here, temperature increases of +3, +6 and $+9^oC$ (above T_0: 24^oC) were applied. We used this broader temperature range during summer in order to reach temperatures (maximal 33°C) which were assumed to be at or above the thermal limit of certain ciliate species. Water temperatures of up to 28^oC can occur in the River Rhine, as e.g. recorded in August 2003. After a ten-day colonisation period, the pioneer biofilm ciliates were analysed by putting the flow cells directly under the microscope. Therefore, a minimum of 60 ciliates per replicate were determined in defined areas by using the identification keys of Foissner and Berger (1996) and Lynn and Small (2002). In the case of colonial ciliates, the number of zooids per colony was added to the recorded abundance of ciliates in the particular replicate. Ciliates were individually measured and their biovolumes were calculated by approximation of the specific shape to standard geometrical forms (e.g. prolate ellipsoid, cone, cylinder).

Based on the observations made in the winter 2006 experiment, we also investigated the impact of simulated winter warming on late, biofilm-dwelling ciliate communities which had reached carrying capacity. In earlier succession experiments, steady-state abundance of biofilm-dwelling ciliates were reached after <9 (summer) to 21 (winter) days (Norf *et al.* 2007). In the present experiments, sterile object slides were exposed in flume systems, starting in November 2007. Three flumes were constantly exposed to untreated Rhine River water (flow velocity 0.2 m s^{-1}): the base temperature treatment (hereafter: T_0), which ranged between 6.1 and 10.2°C. In three additional flumes, the temperature of the bypass of Rhine River water was constantly elevated with heating rods to 3^oC above ambient. After eight weeks, one object slide

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per flume was removed and investigated microscopically. Ciliate identification as well as abundance and biomass calculations were performed as described above.

The first experiment performed in summer (see above) clearly demonstrated a temperature limitation (rather than resource limitation) of the biofilm-dwelling ciliate communities. In one subsequent summer experiment, we tested this hypothesis by simultaneous temperature (T_0 and T_0 + 3°C) and resource level (supplement of planktonic bacteria; see Chapter 3) manipulations. The experiment was conducted in flow cells over ten days in August 2007 as described above. A suspension of *Pseudomonas putida* (MM1) was used in the resource-enrichment treatments according to the protocol described in detail in Chapter 3. For the preparation of the bacterial suspension, kryo-preserved *P. putida* was cultivated for 48 hours at room temperature (20°C) in M9 growth medium (Hahm *et al.* 1994) containing 0.4% glucose. The cultures were harvested by centrifugation (3,600g for 15 min.) and by re-suspending the pellet in Pratt medium (Pratt and Salomon 1980) in order to remove the residual glucose from the cultures. The cultures were then stored at 4°C until needed and were then harvested again as described above. After the cell density of the bacterial cultures had been determined using a Helber counting chamber (W. Schreck, Hofheim, Germany), a suspension containing 10^8 ind. ml^{-1} was prepared in Pratt medium using standard reagent bottles. The bacteria solution was added to one batch of the flow cells by way of sterile silicone tubes. The ratio of bacteria solution to Rhine River water in the flow cells was adjusted to 1:100, so that a final concentration of 10^6 ind. ml^{-1} *P. putida* was fed to the water flow.

Statistical analyses

For the two short-term experiments with experimental temperature increase only (winter and summer 2006, Table 1), we performed one-factorial ANOVAs with temperature as predictor and abundance (ind. cm^{-2}) and biovolume (mm^3 cm^{-2}) as dependent variables using SPSS 15.0 software

(SPSS Inc., Chicago, U.S.A.). The REGW-test was performed as post-hoc test for multiple comparisons. The impact of temperature increase on the abundance and the biovolume of the late biofilm-dwelling ciliate communities which had been cultivated for eight weeks in flumes during winter 2007-08 were compared using the Student T-Test. In order to assess the effects of warming combined with an increased resource availability as observed in the summer 2007 experiment, two-factorial ANOVAs were performed with temperature and resource enrichment used as predictors and abundance (ind. cm^{-2}) or biovolume (mm^3 cm^{-2}) as dependent variables. Temperature-dependent gradients in the taxonomic composition of the biofilm-dwelling ciliate communities for each replicate within the different experiments were analysed by using the quantitative Bray-Curtis similarity index (Bray and Curtis 1957), which was plotted 2-dimensionally using the NMDS ordination method provided in the PRIMER 6.0 software (Primer-E Ltd., Ivybridge, U.K.). The analyses were based on the morphotype abundance data (species and/or genus) as identified under light microscopy. Significant differences between the ciliate communities were detected by the Simprof-test (included in the PRIMER 6.0 software) of the same resemblance data. In the case of significance ($\alpha < 0.05$), the obtained clusters are indicated graphically by circles in the NMDS plots. Rare taxa (with only one or two observations per replicate) were excluded from the analyses in order to reduce noise from the ordinations. In the summer 2007 experiment with simultaneous manipulation of temperature and resource availability, class and sub-class data were used for the calculations of similarities, as species morphotype data was not available for this sample date (day ten).

Results

Warming response of early communities in winter versus summer
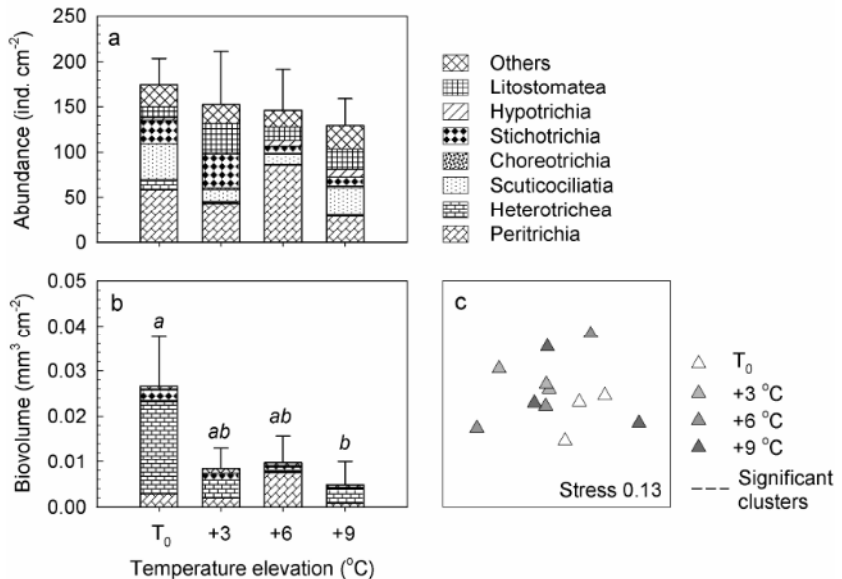
In winter and summer 2006, biofilm-dwelling ciliate communities were cultivated in miniature flow cells at different temperatures starting with the calculated long-term average temperature (T_0) for the particular period. In winter 2006, local

temperature increase resulted in significant differences with regards to the ciliate abundance (Fig. 1a, Table 2) and to the ciliate biovolume (Fig. 1b, Table 2), which was step-like for the two low (T_0, +2°C) and the two high (+4°C, +6°C) temperature treatments. At T_0, ciliate abundance was 320±70 ind. cm^{-2} and did not differ from the ciliate abundance in the +2°C treatment (320±65 ind. cm^{-2}). Ciliate biovolume ranged between 0.005±0.001 mm^3 cm^{-2} (T_0) and 0.007±0.002 mm^3 cm^{-2} (+2°C). Further temperature elevation resulted in both a significantly increased ciliate abundance (750±215 ind. cm^{-2} at +4°C and 700±210 ind. cm^{-2} at +6°C) as well as in a significantly increased ciliate biovolume (0.023±0.015 mm^3 cm^{-2} at +4°C and 0.021±0.010 mm^3 cm^{-2} at +6°C). The observed differences in the ciliate abundances were mainly due to the increased abundances of peritrich (e.g. *Carchesium polypinum*, *Vorticella campanula*), choreotrich (e.g. *Strobilidium caudatum*, *Tintinnidium semiciliatum*) and litostome (e.g. *Litonotus lamella*, *Acineria uncinata*) ciliates (Fig. 1a), which was also reflected in a higher biovolume of these taxa (Fig. 1b).
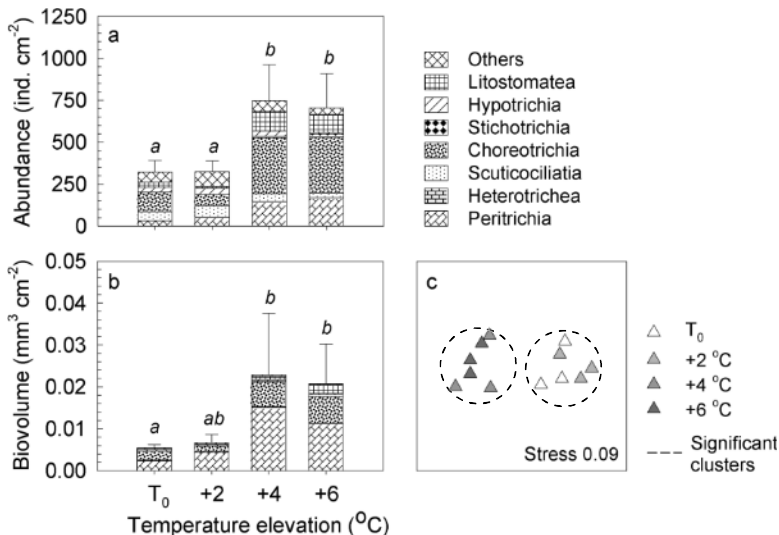
Fig. 1. Impact of temperature increase on the structure of biofilm-dwelling ciliate communities after ten days of succession in winter 2006. (a) Abundance and (b) biovolume of the experimental communities combined with their taxonomic composition. Error bars represent total SD. (c) Non-metric multidimensional scaling (NMDS) visualising the Bray-Curtis similarity of three replicates per temperature treatment. The indicated significant clusters were identified using cluster analysis with SIMPROF test.

Further differences in the taxonomic structures of the biofilm-dwelling ciliate communities were also evident: Calculations of Bray-Curtis similarity indices for each of the replicates and multidimensional scaling of these data produced two significant clusters ($p<0.05$) for the two "low" and the two "high" temperature treatments (Fig. 1c), which corresponded to >40% similarity of the particular ciliate communities. The similarity between all replicates was at least 20%. Different results were obtained in summer 2006. Here, temperature increase did not significantly affect the abundance of ciliates, although a tendency to a reduced abundance was consistently observed (Fig. 2a). In contrast, ciliate biovolume (Fig. 2b) was reduced even by the smallest temperature elevation ($T_0+3°C$). Significant differences appeared between the T_0 treatment ($0.027±0.011$ mm^3 cm^{-2}) and the $+9°C$ treatment ($0.005±0.005$ mm^3 cm^{-2}) as revealed by the one-factorial ANOVA (Table 2, Fig. 2b). This decrease in the biovolume was mainly due to the reduction of large heterotrich ciliates (*Stentor sp.*

Fig. 2. Impact of temperature increase on the structure of biofilm-dwelling ciliate communities after ten days of succession in summer 2006. (a) Abundance and (b) biovolume of the experimental communities combined with their taxonomic composition. Error bars represent total SD. (c) Non-metric multidimensional scaling (NMDS) visualising the Bray-Curtis similarity of three replicates per temperature treatment.

Furthermore, temperature increase did not result in the formation of significant clusters as observed in winter 2006. The Bray-Curtis similarity between all replicates was at least 20% (Fig. 2c).

Table 2. One-factorial ANOVA design for testing the effect of temperature on the abundance (ind. cm-2) and the biovolume (mm3 cm-2) of early biofilm-dwelling ciliate communities in winter 2006 and in summer 2006. **Bold values** indicate significance.

Subject	SS	$F_{(3, 8)}$	p
Winter 2006			
Abundance	486,985	6.708	**0.014**
Within subject	193,600		
Biovolume	0.644	4.923	**0.032**
Within subject	0.349		
Summer 2006			
Abundance	3,238	0.588	0.640
Within subject	204.885		
Biovolume	0.896	0.299	**0.048**
Within subject	0.575		

Warming effects on late communities at carrying capacity in winter

In the next step we tested whether or not the observed responses of the early biofilm-dwelling ciliate communities during winter 2006 were reproducible for late ciliate biofilms at carrying capacity. For this purpose, ciliates were cultivated on object slides exposed in flumes with a permanent bypass of Rhine River water with either ambient (T_0) or increased temperature ($T_0 + 3°C$). After eight weeks, the ciliate abundance in T_0-flumes was 400 ± 50 ind. cm^{-2}. Ciliate abundance was noticeably, although not significantly (T-test: $p=0.11$) higher in the flumes with temperature elevation: 640 ± 150 ind. cm^{-2} (Fig. 3a). The ciliate biovolume was 0.041 ± 0.003 mm^3 cm^{-2} at T_0 and significantly increased to 0.180 ± 0.089 mm^3 cm^{-2} at $T_0+3°C$ (Fig. 3b, T-Test $p<0.05$). The ciliate communities at T_0 consisted mainly of choreotrichs (e.g. *Tintinnidium*
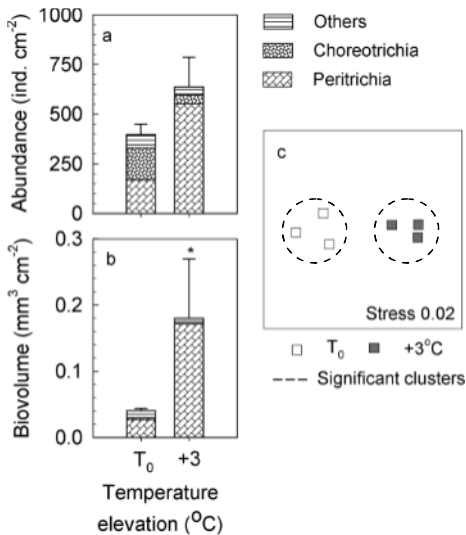
Fig. 3. Impact of temperature increase on the structure of biofilm-dwelling ciliate communities after eight weeks of succession in the flume experiment in winter 2007/ 08. (a) Abundance and (b) biovolume of the experimental communities combined with their taxonomic composition. Error bars represent total SD. (c) Non-metric multidimensional scaling (NMDS) visualising the Bray-Curtis similarity of three replicates per temperature treatment. The indicated significant clusters were identified using cluster analysis with SIMPROF test.

semiciliatum) and peritrichs (*Campanella umbrellaria*, *Carchesium spp*., *Zoothamnium kentii*), in approximately equal proportions (ca. 40% each) in terms of abundance. Choreotrichs were reduced to less than 10% of the ciliate abundance in the flumes with temperature elevation (Fig. 3a), particularly due to a strong decrease in the number of *Tintinnidium semiciliatum*. The ciliate biovolume (mm^3 cm^{-2}) in both setups consisted mainly of peritrichs, which accounted for <65% at T_0 and approximately 90% in the flumes at $T_0 + 3°C$. The analysis of the taxonomic compositions of the biofilm-dwelling ciliate communities on the basis of their Bray-Curtis similarity revealed two significant clusters ($p < 0.05$) for the two temperature treatments, which corresponded to >60% similarity of the communities at each temperature and >40% similarity between the two temperatures (Fig. 3c).

Temperature versus resource effects in summer

In the last experiment we simultaneously manipulated the temperature and the resource level in order to test the significance of the resource level on community responses towards warming during summer, after the preceding summer experiment (2006) had revealed significant negative warming effects

on the biovolume. Therefore, a suspension of planktonic bacteria (*P. putida*, an important resource for biofilm-dwelling ciliates in the Rhine) was added to the flow cells, resulting in four different setups: Flow cells with either increased temperature (T_0 + 3°C) and/or increased food levels. Both the enhanced temperature as well as the resource enrichment resulted in significant impacts on either the abundance or the biovolume of biofilm-dwelling ciliates (Fig. 4). In the setups with no supplemental resource added, warming resulted in a significant decrease in the ciliate abundance by approximately 25% and in a non-significant decrease in the ciliate biovolume. The flow cells which obtained the resource enrichment displayed a tendency towards increased ciliate abundance. The ciliate biovolume was increased by more than 100% in the flow cells with resource enrichment only, whereas simultaneous resource enrichment and temperature increase resulted in a lower ciliate biovolume than when only the food supply was increased.
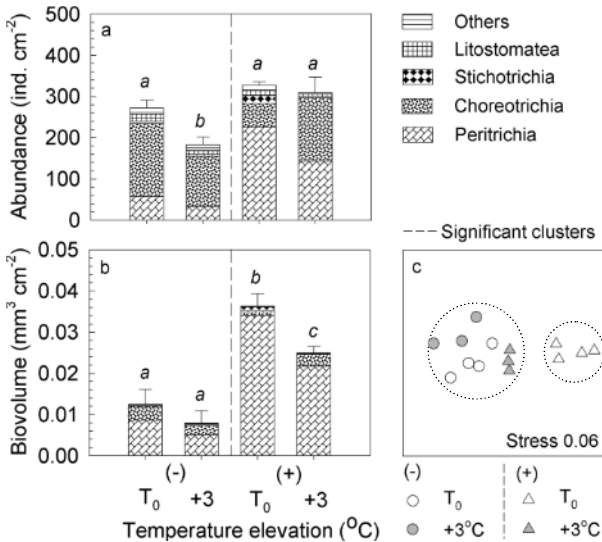
Fig. 4. Impact of temperature increase and resource addition (as indicated by "+" in contrast to the ambient resource conditions as indicated by "-") on biofilm-dwelling ciliate communities tested after ten days of succession in summer 2007. (a) Abundance and (b) biovolume of the experimental communities combined with their taxonomic composition. Error bars represent total SD. (c) Non-metric multidimensional scaling (NMDS) visualising the Bray-Curtis similarity of three to four replicates per treatment. The indicated significant clusters were identified using cluster analysis with SIMPROF test.

This was, however, still significantly higher than in the flow cells with no resource enrichment. The two-factorial ANOVAs revealed a significant impact of *temperature* ($p<0.05$) and *resource enrichment* ($p<0.001$) on both the abundance as well as on the biovolume of the biofilm-dwelling ciliates (Table 3). Furthermore, there was a significant interaction *temperature x resource enrichment* ($p<0.05$) detected for the abundance of the ciliates. In terms of abundance, choreotrichs (e.g. *Strobilidium caudatum*, *Tintinnidium semiciliatum*) and peritrichs (e.g. *Carchesium polypinum*, *Vorticella* spp.) were dominant in all treatments. Remarkable differences appeared with the flow cells with resource enrichment; the abundance of peritrich ciliates was strongly increased, whereas choreotrich ciliates were reduced, especially in the flow cells which were run at T_0. These effects were also detected with regards to the biovolume of the biofilm-dwelling ciliate communities. Calculation of the Bray-Curtis similarities revealed two significant clusters. The first cluster included the two treatments with no resource enrichment as well as the treatment with cross manipulation. The similarity between these communities was >60%.

Table 3. Two-factorial ANOVA design for testing the effect of temperature and resource enrichment on the abundance (ind. cm-2) and the biovolume (mm3 cm-2) of early biofilm-dwelling ciliate communities in summer 2007. **Bold values** indicate significance.

Subject	SS	F (1, 8)	p
Abundance			
Temperature	8,533.333	6.708	**0.004**
Resource enrichment	24,661.333	45.833	**<0.001**
Temperature x resources	3,792.593	7.048	**0.029**
Error	4,304.593		
Biovolume			
Temperature	191.179	22.721	**0.001**
Resource enrichment	1,249.624	148.517	**<0.001**
Temperature x resources	34.992	4.159	0.076
Error	67.312		

The second cluster was made up of the four replicates with resource enrichment which had been cultivated at T_0 with also >60% similarity between the replicates of this treatment. Significant temperature effects on the community structure were thus obtained in the resource-enriched but not in the non-enriched treatment. The similarity between all performed replicates was at least 40%.

Discussion

In the present work we implemented an experimental approach to test the impact of environmental warming on complex consumer communities using fast-growing ciliate communities (Jessup *et al.* 2004; Weisse 2006). The river bypass systems ensured the opportunity for both ciliate immigration and emigration and thus acknowledged the open nature of local communities (Leibold *et al.* 2004). We tested the effect of warming in contrasting seasons (summer versus winter), incorporating different background conditions. The growth of ciliate communities in the River Rhine can be resource limited during summer, whereas indications for temperature limitation during winter have been found in earlier studies (Scherwass and Arndt 2005; Norf *et al.* 2007). The present results in fact revealed opposing effects of warming on ciliate communities for summer and winter. In the following, mechanisms leading to the warming effects for the winter and summer experiments are discussed separately before a general conclusion is drawn.

Winter warming alters ciliate community structure and stimulates their total density

The two winter experiments revealed consistently positive effects of warming on the quantity (abundance and biovolume) as well as significant effects on the community structure. Nevertheless, different mechanisms might be involved in the early (ten days, flow-cell experiment) and in the late (eight weeks, flume experiment) experiments. According to our long-term experience in the

establishment of biofilms in river bypass systems (Weitere *et al.* 2003; Norf *et al.* 2007), biofilm-dwelling ciliate communities achieve a steady state in abundance after approximately 21 days during winter and after fewer than nine days during summer. The early biofilms in winter were thus in a stage of increasing abundances, whereas the late biofilms in winter (as well as the biofilms in the summer experiments) already had reached carrying capacity. In a previous study (Norf *et al.* 2007) we demonstrated different effects of warming on the abundance of early versus late biofilm-dwelling ciliate communities. In early communities, warming led to a stimulation of the abundance (probably due to stimulated growth), whereas warming in late communities at carrying capacity resulted in negative effects on the abundance. The latter effect was weak, and no seasonal dependencies could be identified. However, it was in accordance with the hypothesis that carrying capacity decreases with warming in order to balance increasing metabolic costs (Savage *et al.* 2004). The present data rejects this hypothesis for the winter communities, for which significant stimulating effects of warming on the biomass were found whereas positive trends (but non-significant), were shown for the abundance. These quantitative effects were accompanied by significant effects on the community structure with non-symmetrical temperature responses of specific taxonomic groups: Whereas suspension-feeding peritrich ciliates were strongly enhanced by the temperature manipulation, choreotrich ciliates (with similar feeding preferences as peritrichs) were strongly reduced in these setups. Since choreotrichs were still present and since the temperature increase was only 3°C, it is unlikely that the temperature tolerance of the choreotrichs present was exceeded, but rather that choreotrichs (which showed positive responses to enhanced temperature in the early winter biofilms in the flow-cell experiment) were out-competed by peritrichs in the late ciliate communities. On this account, Jiang and Morin (2004) reported that the interaction strength of species within a community can be altered by temperature fluctuations. The significant finding in this study is that the shifts in the community structure coincide with changes in the quantity; the peritrichs were significantly larger

than the choreotrichs and occurred in slightly higher densities. Peritrichs have stalked cell bodies, which probably allow them to take advantage of additional resources in higher layers of the water column. The data thus suggest that competitive exclusion due to warming can alter the density by the establishment of species with different properties with respects to both food exploration and density limitations. Such mechanisms in complex communities can lead to other effects of warming on the total density than those suggested by the applications of populations models rooted in metabolic theory (Gillooly *et al.* 2001; Savage *et al.* 2004).

The positive effects of warming on both the abundance and on the biovolume in the early winter communities were probably due to stimulated growth at sufficient resource supply (Scherwass *et al.* 2005; Norf *et al.* 2007). However, it is remarkable that the stimulation of ciliates occurred stepwise between the two "low" (T_0, $+2^{o}C$) and the two "high" ($+4$, $+6^{o}C$) temperatures, whereas a homogenous growth stimulation would suggest a continuous increase (Weisse *et al.* 2002). The effects on the quantity were again accompanied by a clearly differentiated community structure, as demonstrated with the help of the Bray-Curtis similarity of the ciliate abundances. Remarkably, no intermediate cluster occurred in response to the stepwise warming. It seems thus that the ciliate communities were initially buffered against small changes in temperature (T_0 vs. $+2^{o}C$) as has been discussed for other natural communities (Jiang and Kulczycki 2004). If this temperature range is exceeded, however, small temperature changes can lead to rapid changes in the community structure.

Negative warming effects on community density are coupled to low resource availability during summer

The two summer experiments focussed on different aspects of the effects of warming on the community composition. In the first experiment, the temperature was increased in four steps up to 33°C, which is distinctly higher than the maximal temperatures thus far recorded in the River Rhine (ca. 28°C). As this experiment revealed negative effects on the ciliate quantity (significant

for the ciliate biovolume), the second experiment was designed to determine whether or not these negative warming effects could be compensated by resource supplement, as has been suggested to be the case when the negative effects are due to increased metabolic costs (Laybourn and Finlay 1976; Savage *et al.* 2004). The alternative mechanism, i.e. that the warming effects are mainly due to thermal limitations of the different species, would imply that the effects cannot be compensated by resource supplement.

Both summer experiments consistently show that warming above the summer ambient conditions (and without resource supplement) has negative effects on the total ciliate density, a result which stands in contrast to the findings of the two winter experiments. The temperature range of the first summer experiments was chosen to exceed thermal tolerance of many ciliate species. It was a surprising finding that no taxa consistently disappeared at higher temperatures. Thus, the lack of significant community effects could partly be due to the large variability and thus an overlay of temperature effects with random effects as indicated by a rather low (<40%) Bray-Curtis similarity. We also do not know whether or not cryptic responses beyond the taxonomic detection level occurred. An increasing number of studies highlight the existence of various geno- and ecotypes beyond the morphospecies level (Barth *et al.* 2006; Weisse and Rammer 2006). The frequency of such types can change in response to environmental factors, a mechanism which can stabilize the total morphotype abundance (Meyer *et al.* 2006; Yoshida *et al.* 2007). Such possibilities have been discussed for mesocosm experiments in which a temperature increase had driven microevolutionary responses in *Simocephalus vetulus* that buffered the total population of this zooplankter against environmental change (Van Doorslaer *et al.* 2007). Such mechanisms could be one reason for the lack of a significant response of the community structure towards warming.

Nevertheless, it is still remarkable that clear indications for thermal limitations of morphotypes were lacking and that high temperatures did not lead to obvious shifts in the functional composition. The results of the resource supplement in the second summer experiment supported the conclusion that the summer

community was resource rather than temperature limited. The resource supplement compensated the negative warming effects on the total abundance and thus supported the conclusion that increasing metabolic costs at limited resource level are the main reason for the negative warming effect in the natural setting (Savage *et al.* 2004). This shows that resource availability rather than temperature limitation determines the quantity of ciliate summer communities, in contrast to the observations during winter, in which (low) temperature limitation rather than resource availability constrained the biofilm-dwelling ciliate communities. Nevertheless, we also found effects on the community composition which coincided with significant warming effects on the biomass (but not on the abundance) for the treatments with supplemented resources. As discussed for the winter experiments above, such community shifts make the application of population models to the complex community difficult. In contrast to the winter effect, however, the biovolume generally decreased with warming in summer. The reason is probably metabolic cost: Laybourn and Finlay (1976) found ciliate growth to be constrained by temperature enhancement as a consequence of large respiratory energy losses. These losses increase dramatically with increasing body size. This could possibly explain the generally decreasing body mass in summer and that especially the number of large heterotrich ciliates was reduced in the summer 2006 experiment.

It is interesting that the communities established under warming plus resource supplement resembled the communities established without supplement (both warmed and non-warmed) whereas all three significantly differed from the community in the supplemented, non-warmed treatment. This finding supports the conclusion of a strong relationship between temperature and resources on the community level in that warming during times of resource surplus results in similar effects as resource limitation. Together with the results from the winter experiment, it further shows that communities are more likely to be affected by warming if resources are not limited.

Conclusion

The present results emphasize the significance of the environmental conditions when considering community responses towards warming. Particularly the resource availability and the temperature-specific resource demand determine whether or not a temperature increase alters population growth or carrying capacity. Nevertheless, the community response towards warming can differ considerably from the responses of populations, as temperature increase can alter the community composition and thus favour the establishment of species with different properties regarding density regulation. The predictive value of population models for changes in the community density is thus limited.

CONTROL OF CILIATE COMMUNITIES BY RESOURCES

Chapter 3.

Control of early ciliate communities by resources: Impact of planktonic and benthic resource supplements.

Abstract

Four seasonal experiments were performed to test the impact of increased benthic and planktonic resource availability on the structure of biofilm-dwelling ciliate communities which were cultivated in river bypass systems. The growth of benthic bacteria was stimulated by the addition of dissolved organic carbon and the enrichment of the planktonic resource was achieved by supplementation with suspended bacteria. It was shown that both resource enrichments can differentially influence abundance and taxonomic structure of the consumer communities. Furthermore, both resources were found to influence different stages during biofilm colonization. Increased benthic bacterial growth mainly resulted in both an accumulation of primarily grazing-resistant bacterial filaments and in an increase in the number of vagile heterotrophic flagellates. The effect on ciliates was an indirect stimulation of nanophagous ciliates (feeding mostly on the flagellates), rather than a direct stimulation of bacteriovorous ciliates. The effects of the planktonic bacteria enrichments were twofold: They could have been utilized either directly by suspension-feeding ciliates or indirectly through an enhanced growth of suspension-feeding attached heterotrophic flagellates, which were then in turn grazed upon by ciliates. The magnitude of responses of the total ciliate abundance to the two resource enrichments further depended on the seasonal conditions, thereby showing seasonally variable limitations of these resources. Furthermore, the particular taxonomic groups stimulated by one resource type

sometimes differed depending on the season, an observation which demonstrates that the response depends on different environmental factors and is not easily predictable based simply on resource type. Taken together, our results emphasize the need of a differentiated view on the effects of resources on complex biofilm-dwelling consumer communities with respect to both the origin of carbon source as well as the particular environmental conditions.

Introduction

Microbial assemblages on surfaces play an important role in the metabolic processes of both marine and freshwater environments. Especially in running waters, the major part of microbial activity is located in association with submerged surfaces (Bryers and Characklis 1982; Fischer *et al*. 2002). Besides consisting of algae and bacteria as primary producers, these so-called biofilms are also inhabited by a variety of consumers, in particular ciliates, which make up a large portion of the total biofilm biomass (Finlay and Esteban 1998). As ciliates can prey on a wide size spectrum ranging from bacteria to small micrometazoans (Parry 2004), they are likely to be among the most important mediators for energy- and matter flux within microbial biofilms.

However, the effective function of biofilm-dwelling ciliate communities largely depends on their structure, both in terms of abundance as well as their taxonomical and functional composition. As ciliates can rapidly react to changes in their environment because of their high individual growth rates (Müller and Geller 1993) as well as a large local species pool (Andrushchyshyn *et al*. 2003; Müller *et al*. 1991), their community structure can be rapidly affected by changes in any of several abiotic and biotic factors (Andrushchyshyn *et al*. 2003; Gong *et al*. 2005; Primc-Habdija *et al*. 2005). Ciliate communities are particularly affected by resource availability, as has been shown in several field studies on planktonic (Andrushchyshyn *et al*. 2006; Scherwass and Arndt 2005; Wiackowski *et al*. 2001) and biofilm-dwelling (Gong *et al*. 2005, Wickham *et al*. 2004) ciliate assemblages. As inhabitants of interfaces, biofilm-dwelling ciliates have potential access to both suspended as well as surface-associated particles, and the successful utilization of these resources largely depends on their specific feeding mode (Parry 2004). The ability to consume suspended particles is mainly found in ciliate taxa which can produce a water current by the movement of either their apical cilia (e.g. peritrichs), of outstretched membranelles (e.g. scuticociliates, hymenostomes) or of adoral membranelles (e.g. heterotrichs, stichotrichs, hypotrichs and choreotrichs). Other ciliate taxa, which mainly crawl over

the substrates, feed on surface-associated particles such as algae, different bacterial morphs or flagellates, by the use of "food baskets" (e.g. cyrtophores, nassophores) or specific oral clefts (e.g. litostomes, prostomes). These differences in the feeding morphologies of ciliates suggest that each taxon has specific demands regarding its resource. However, many taxa are known to feed on more than one prey, and even suspension-feeding ciliates can actually utilize benthic resources (Parry 2004). Despite their pronounced contribution to ecosystem function, there is still a lack of case studies which document the contribution of the two resource types (suspended versus surface-associated) in structuring natural consumer communities within biofilms.

Two different experimental approaches are mainly utilized to investigate the coupling between communities and their resources. Laboratory experiments which allow the direct and standardized manipulation of food sources mainly focus on the process of resource uptake and growth responses of cultured ciliates to different nutrient levels. Species usually respond positively to increased resource availability, which results in higher feeding and growth rates. However, the transposition of laboratory results to complex field communities is sometimes problematic, as similar experimental setups in the laboratory and in the field can produce different results, as shown for the response of peritrich ciliates to experimentally increased sedimentation (Bergtold and Traunspurger 2005). Field studies are mainly used to investigate the value of bottom-up factors in controlling complex field communities. They have already demonstrated the likely importance of the availability of resources especially for planktonic ciliate communities by correlating the environmental setting to ciliate abundances and community identities (Carlough and Meyer 1989; Gong *et al.* 2005; Kisand and Zingel 2000, Müller *et al.* 1991). These suggestions were taken up in some manipulative field studies, in which the resource level was indirectly stimulated by fertilization in order to enhance the growth of prey (e.g. bacteria, algae) for ciliates. However, the results of these studies are oftentimes controversial and strongly differ depending on the site, season, and duration of the observation. For example, Hillebrand *et al* (2002)

found some weak effects of supplemental nutrients in lake mesocosms on bacteria, heterotrophic flagellates and ciliates, whereas Wilcox *et al* (2005) observed significant effects of labile carbon addition to the headwater of a stream on bacteria and metazoans, but no effect on heterotrophic flagellates and ciliates. Hence, a mechanistic understanding on the effects of resources on biofilm consumer communities can only be accomplished by direct manipulation of the resource level and by investigations with high temporal resolution.

The aim of this study was to test the effect of increased resource availability on the structure of semi-natural, biofilm-dwelling ciliate communities and to determine the pathways through which resources can be acquired by these communities. Using two different resource enrichments, we focused on (1) the response of ciliates to indirect resource manipulations by stimulating the growth of benthic bacteria with DOC and (2) the response of ciliates to direct enhancement of planktonic bacteria by permanent addition of pre-cultured bacteria to the experimental facilities. Our hypothesis was that both resource types significantly alter the ciliate community structure, although in different ways. The DOC enrichment should indirectly stimulate the growth of biofilm-dwelling ciliates due to an enhanced growth of benthic bacteria and thereby also of vagile heterotrophic flagellates as consumers of the bacteria. The enhancement of the planktonic bacteria, in contrast, was expected to directly stimulate the growth of ciliate taxa which can efficiently collect suspended particles from the water flow (e.g. peritrichs, scuticociliates). The experimental setup was designed to combine the advantages of laboratory studies (standardized experimental conditions and community analyses with high temporal resolution) and those of field studies (utilization of natural ciliate assemblages). This was achieved by growing and experimentally manipulating biofilms in flow cell systems connected to river water as a source of biofilm-colonizers and ambient resources supply.

Material and Methods

Study site and experimental setup

All experiments were performed aboard the Ecological Rhine Station of the University of Cologne (Cologne, Germany, Rhine-km 684.5, which refers to the distance from Lake Constance, the source of the non-alpine Rhine) – a moored laboratory ship that is located in the main stream of the River Rhine. The primary tool of all experiments were miniature flow cells which were adapted from laboratory experiments (Stoodley *et al.* 2001) and now can also be used for the cultivation and non-destructive live observation of field-related biofilm communities (Norf *et al.* 2007). The flow cells consist of a plastic chamber with the size of a standard object slide, enclosing a space of 3.6 ml volume. This area is enclosed by an object slide on the bottom and a cover slip on the top. The flow cells are then fed with fresh Rhine River water through miniature tubes at the short flow cells margins using impeller pumps. The utilization of original Rhine River water provided the species-pool for biofilm colonization as well as the ambient nutrient supply. This ambient supply in the natural water was then further supplemented with additional resources (see below). The flow cells are designed to fit on a standard cross table of a microscope. A more detailed description of the flow cells used here is given by Norf *et al* (2007).

Experimental setup and culturing methods

The experiments described hereafter were conducted during four periods in 2006 and 2007, covering four different seasons (hereafter: fall 2006, winter, spring and summer 2007). All experimental treatments were performed in triplicate. Temperature was logged on every observation date with a digital thermometer. The ambient settings for the experiments including planktonic bacterial and algal abundances as well as DOC concentrations are given in Table 1. Planktonic bacterial (DAPI-counts) and data on algal abundances were kindly provided from routine observations by Marcel Kathol and Maria Gies (University of Cologne). Information on DOC amounts were kindly provided by the Gas- und Elektrizitätswerke Köln (GEW, Cologne, Germany).

Two different experimental designs were applied in each season to test the impact of enhanced benthic and planktonic resource availability on developing biofilm-dwelling ciliate communities, starting from sterile (autoclaved) surfaces. Both designs were run in parallel, thus allowing cross-comparisons. The first design accounted for the effects of stimulated benthic bacterial growth on the development of ciliate communities by adding DOC in the form of yeast extract to the flow cells (hereafter: DOC ENR); a second setup did not obtain any supplemental resource and thus only contained the ambient resources of the Rhine water (hereafter: AMB). For preparation, 0.5 mg l^{-1} yeast extract (Sigma-Aldrich Co.) were suspended in particle-free Rhine River water and autoclaved. The suspension was then fed into the flow cells with impeller pumps and sterile miniature silicone hoses. The DOC solution was diluted 1:100 with original Rhine River, resulting in a final concentration of 0.005 mg l^{-1} yeast extract within the flow cells. This concentration was shown to sufficiently enhance - but not exceed - the bacterial growth within the flow cells in pre-experiments. A slightly higher concentration (0.01 mg l^{-1}) resulted in excessive bacterial growth and associated oxygen depletion within the flow cells after approximately one week, making a detection of the carrying capacity for ciliates impossible (Norf *et al.* 2007).

Table 1. Ambient settings of selected environmental variables in the River Rhine during the seasonal experiments. The given values are the mean values (±SD).

Season	Duration	Temp. (ºC)	DOC (µg ml^{-1})	Plankt. bacteria (10^6 cells ml^{-1})	Plankt. algae (cells ml^{-1})
2006					
Fall	Oct 30. -Nov 13.	12.0±0.6	2.9±0.1	0.9±0.2	450±85
2007					
Winter	Feb 1.-15.	7.0±0.4	2.8±0.5	1.7±0.2	718±176
Spring	Apr 24. - May 6.	19.2±0.7	2.2±0.1	2.6±0.6	13,006±2,120
Summer	Aug 23. - Sep 2.	19.7±0.9	2.7±0.2	1.6±0.2	967±60

The second setup was designed to help determine the influence of suspended bacteria as one planktonic prey on the development of biofilm-dwelling ciliate communities. Therefore, a suspension of vital non-toxic bacteria (*Pseudomonas putida*, MM1; Dütz *et al.* 1994) was fed into the flow cells (hereafter: BAC ENR). *P. putida* is a common bacterium that has often been shown be an optimal food for bacteriovorous ciliates (Eisenmann *et al.* 1998; Tso and Taghon 1999). A second treatment was supplied with a filtrate (0.2µm) of the bacterial culture (hereafter: BAC CON), which served as the baseline for the BAC ENR to distinguish the effects of the suspended prey (bacteria) from possible effects of leaching products on the experimental biofilms. For preparation, kryo-preserved *P. putida* were cultured in 50% M9 culture medium (Hahm *et al.* 1994) containing 0.04 g l^{-1} glucose at room temperature (20°C). The cultures were harvested by centrifugation at 3,400 g after two days. This treatment of the bacteria was shown to reduce the ability of biofilm formation with *P. putida* (Bell *et al.* 2005). Afterwards, the pellet was suspended in 50ml Pratt medium (Pratt and Salomon 1980) - a minimal culture medium with low osmolarity - in order to remove the residual glucose from the cultures. The cultures were then refrigerated (+4ºC) for two days and then harvested by centrifugation as described above. After the cell density was determined by the use of a Helber counting chamber (W. Schreck, Hofheim, Germany), a suspension containing 1×10^8 ind. ml^{-1} (final abundance) was prepared in 500 ml Pratt medium using standard reagent bottles. The two solutions (bacteria and filtrate) were added to the BAC ENR and the BAC CON treatments with sterile silicone hoses. Because the ratio of bacteria solution to field water in the flow cells was 1:100, the final concentration of *P. putida* in the flow cells was 10^6 ind. ml^{-1}. This concentration of bacteria was chosen in order to significantly enhance the abundance of the naturally occurring planktonic bacteria (which was determined to be between 0.9 and 2.6 x 10^6 ind. ml^{-1}, see Table 1). The bacteria suspensions were kept in a water bath at 6°C during the experiments and were renewed every two days.

Data analysis

The development of the microbial biofilms was tracked for at least ten days, starting from sterile surfaces. Four categories of organisms were included in the surveys, with ciliates being the major subject of the experiments. Therefore, the early succession of biofilm-dwelling ciliates was tracked for at least ten days by examining an area with a minimum of 60 individuals using a microscopic grid, which was repeatedly placed randomly at different spots of the flow cell. The taxonomic composition of the present ciliates was recorded on the basis of taxonomic units (either class or subclass) using standard literature (Foissner and Berger 1996). After completion of the experiments, two different measures were taken in order to estimate both the colonization speed and the carrying capacity. We utilized regression models in order to calculate the time-span (t_{50}) until 50 ind. cm^{-2} had colonized the inner flow cell surfaces. The exponential model used was $A = a*e^{b*time}$ with A being the ciliate abundance (ind. cm^{-2}). The two variables a and b were calculated by iteration using the SPPS 15.0 software (Statcon Ltd.). This procedure was similar to that performed for temperature manipulation experiments with biofilm-dwelling ciliate communities as described in Norf *et al* (2007). The regression estimates thus obtained were solved with a theoretical ciliate abundance of $A = 50$ ind. cm^{-2} in order to estimate the time span until 50 ciliates cm^{-2} had populated the inner flow cell surfaces (t_{50}). The maximum observed abundance of biofilm-dwelling ciliates as an estimate for the carrying capacity was extracted directly from the experimental raw data for each treatment and replicate, irrespective of the date on which this abundance was observed. Both measures, the calculated factor t_{50} and the empirically determined maximal abundance of ciliates, were compared pair wise using the Student T-Test: The AMB treatment (no resource added) served as the baseline for the flow cells which obtained the DOC enrichment (DOC ENR). The filtrate of the bacterial culture (BAC CON) provided the baseline for the flow cells which obtained the supplemental pre-cultured bacteria (BAC ENR) to separate the pure effects of the fed bacteria from possible effects of leaching products of the bacterial cultures.

In the following analyses, we focussed on differences in the succession pattern due to resource enrichment during the experimental phase. Therefore, repeated measures ANOVAs (rmANOVAs) were calculated with <u>time</u> as the inner-subject factor and <u>treatment</u> (resource enrichment) as the between-subject factor. Using the rmANOVA we tested for effects of enhanced resource availability on both the total ciliate abundances as well as on specific taxonomic units (either classes or subclasses). All analyses were performed as pair wise comparisons (AMB vs. DOC ENR and BAC CON vs. BAC ENR). In addition to the ciliates, we also tracked the number of bacterial filaments, heterotrophic flagellates (hereafter: HF) and rotifers as concomitant parameters on representative days within the experiments. Therefore, the abundance of bacterial filaments (>20µm) and HF was determined for an area of 0.05 cm^2 using a microscopic grid at 200x magnification. We also calculated the biovolume of the bacterial filaments by measuring each filament (length, diameter) and considering the filaments to be cylindrical in shape. The HF abundance was recorded for vagile (motile) and sessile HF separately and was used to check for significant differences in the succession patterns using rmANOVA as described above. Rotifers were generally represented in low quantities. A larger number of rotifers, which was expected to contribute to the biofilm dynamics, was observed only in spring 2007. Here, the abundance of rotifers was determined in a representative area of approximately 0.5 cm^{-2} using a microscopic grid at 50x magnification.

Results

The experimental biofilm-dwelling microbial communities exhibited a highly seasonally-dependent pattern in terms of abundance, taxonomic composition and response towards the resource manipulations. Besides the detailed investigation of the present ciliate communities, we also recorded and analyzed additional data for HF as possible ciliate prey, bacterial filaments as a (though

not fully) grazing-resistant morphotype and for rotifers as possible consumers of ciliates.

Growth of bacterial filaments within the flow cells

The development of bacterial filaments within the flow cells was recorded for all experiments and treatments. These filaments were quantified in winter, spring and summer 2007. The maximum bacterial filament biovolume was rather low in the AMB flow cells, ranging from 0.04 to 0.06 x 10^7 μm^3 cm^{-2} (Fig. 1); the addition of DOC (treatment DOC ENR) significantly increased the biovolume in all experiments (Fig. 1, Table 2), with the strongest impact found in winter 2007 (max. 2.4 x 10^7 μm^3 cm^{-2}). A tendency towards enhanced bacterial growth was often recorded in the BAC CON flow cells, in contrast to the BAC ENR flow cells. However, the only significant increase in bacterial filament volume in the BAC CON treatment appeared in summer 2007 (Fig. 1, Table 2).

Responses of heterotrophic flagellates (HF) to resource enrichments

HF abundances were recorded separately for vagile HF (e.g. the mostly benthivorous genera *Neobodo*, *Rhynhomonas* and *Ancyromonas*) and sessile HF (e.g. the mostly planktivorous genera *Monosiga*, *Codonosiga* and *Anthophysa*) in order to distinguish possible effects of enhanced benthic and planktonic resource availability on these two different HF groups.
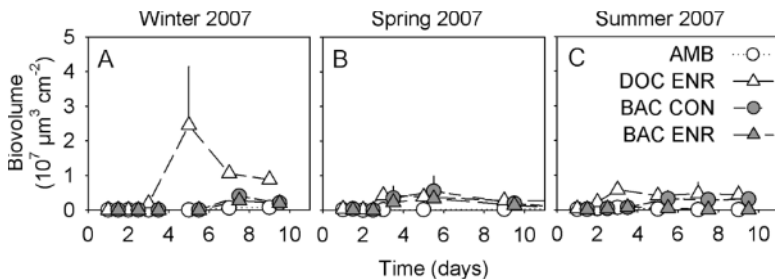
Fig. 1. Biovolume (μm^3 cm^{-2}) of bacteria filaments (>20µm length) which developed in the flow cells during the experiments. (A) winter 2007, (B) spring and (C) summer 2007. The symbols for the DOC ENR and the BAC ENR experiments were separated by 0.5 days on the x-axis for optical purposes, even though they refer to the same sample dates. Error bars (for optical reason in one direction only) represent SD.

Vagile HF abundances generally increased rapidly within the first days of succession in all treatments, whereas the development of the sessile HF communities was always delayed. The maximal abundance of vagile HF varied between 1,000±200 (fall 2006) and 2,100±400 ind. cm^{-2} (winter 2007) in the AMB treatments. The abundance was significantly increased by DOC ENR (Table 2) in all four experiments, resulting in maximal abundances of between 1,600±500 (fall 2006) and 3,600±800 (winter 2007) ind. cm^{-2}. In the BAC ENR treatment, the maximal observed abundances of vagile HF ranged between 1,600±500 (spring 2007) and 5,800±2,000 (fall 2006) ind. cm^{-2}. Although this vagile HF abundance in fall 2006 was the highest recorded during the experiments, there was no significant difference between this value and the BAC CON baseline (Table 2); the only significant difference between the BAC ENR and the BAC CON treatments for vagile HF appeared in winter 2007 (rmANOVA: treatment, $p<0.05$). Maximal sessile HF abundances ranged between 3,500±650 (summer 2007) and 9,900±3,500 (spring 2007) ind. m^{-2} in the AMB treatments and were not affected by the DOC ENR (Fig. 2, Table 2). The BAC ENR resulted in a strongly significant increase of sessile HF in comparison to BAC CON (Table 2) to maxima between 7,300±3,200 (summer 2007) and 54,500±15,800 (spring 2007) ind. cm^{-2} (Fig. 2)
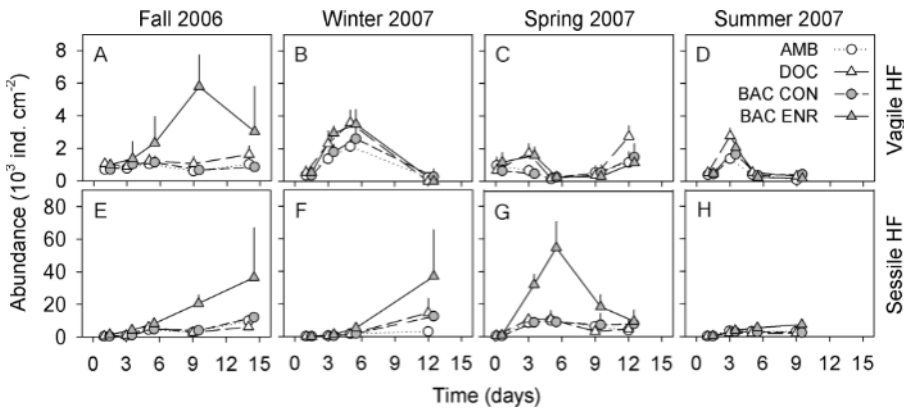


Fig. 2. Abundance of heterotrophic flagellates (HF). The total abundance of HF was split into (first row, A-D) vagile HF and (second row, E-H) sessile HF. The symbols for the DOC ENR and the BAC ENR experiments were separated by 0.5 days on the X-axis for optical purposes, even though they refer to the same sample dates. Error bars (for optical reasons in one direction only) represent SD.

In spring 2007, however, sessile HF abundance was observed to decrease rapidly after initially peaking on day 5. In all other experiments, sessile HF accumulated towards the end of the experiments.

Impact of resource enrichment on the density of biofilm-dwelling ciliate communities

We attempted to partition possible effects of resource enrichments on ciliate communities by concentrating on possible differences within the earliest phase of succession as well as on the maximum abundance of ciliates. Using non-linear regressions, we first calculated the time span until 50 ciliates cm^2 had colonized the inner flow cell surfaces. This factor t_{50} (Fig. 3A) varied greatly with the season with regards to the AMB treatment, with the fastest colonization (lowest t_{50}) observed in spring 2007 (2.2 ± 0.1 days) and the slowest colonization (highest t_{50}) in winter 2007 (7.4 ± 0.1 days). The impact of the DOC ENR on t_{50} was generally low. Though a tendency to faster succession was always observed, the only significant reduction of t_{50} was found in summer 2006 (T-Test, $p<0.05$). The BAC ENR treatment always resulted in a significant reduction of t_{50} (T-Test, $p<0.05$).Analogous to the high seasonal variation in the
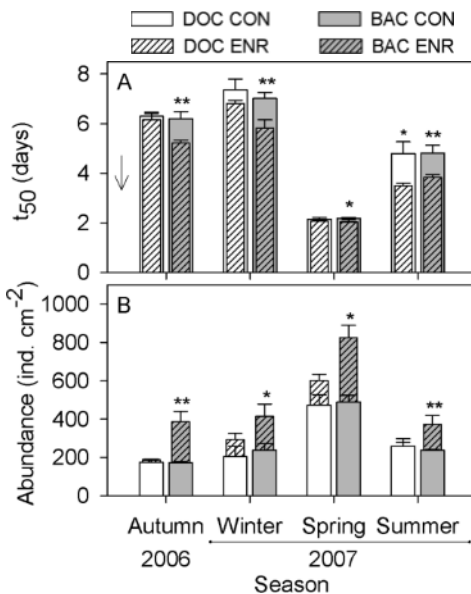
Fig. 3. Impact of resource enrichment on two selected variables during the early development of biofilm-dwelling ciliate communities. (A) Colonization speed t_{50}, as determined by non-linear regressions. A lower value in t_{50} means faster succession, as indicated by the direction of the arrow. (B) Maximum abundance (ind. cm^{-2}) of ciliates observed during the experiments. Asterisks (*) indicate significant differences between the treatments (T-Test, *$p<0.05$, **$p<0.01$). Error bars represent SD.

colonization rate, there were also large differences found in the maximum ciliate abundance (Fig. 3B). The highest maximum abundance regarding the AMB setting was observed in spring 2007 (470 ± 50 ind. cm^{-2}). The lowest maximum ciliate abundance appeared in fall 2006 (180 ± 10 ind cm^{-2}). At no time did the DOC ENR result in a significant increase in the maximum ciliate abundances compared to the AMB treatment. In contrast, BAC ENR always resulted in a maximum ciliate abundance significantly (T-Test, $p<0.05$) higher than that of the BAC CON baseline. Here, the strongest enhancements of ciliate maximum abundances were recorded in fall 2006 (390 ± 50 vs. 170 ± 10 ind. cm^{-2}) and in spring 2007 (830 ± 60 vs. 490 ± 30 ind. cm^{-2}).

Table 2. Results of the repeated measurement ANOVA (rmANOVA) design to test for significant effects of resource enrichments on the biovolume of bacterial filaments and abundance of mobile and attached HF as well as ciliates in pair-wise comparisons (AMB vs. DOC ENR and BAC CON vs. BAC ENR). Values represent F-ratios and *asterisks* indicate significance (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$).

		Time	Treatment	Time x Treatment
		$F_{(1, 4)}$	$F_{(7, 28)}$	$F_{(7, 28)}$
AMB vs. DOC ENR				
Bacteria	fall 2006	-	-	-
	winter 2007	4.09*	109.11***	5.13*
	spring 2007	1.32	39.62***	3.80*
	summer 2007	7.82*	21.06**	5.36*
HF (vagile)	fall 2006	9.81***	9.34*	0.52
	winter 2007	26.39***	13.27*	1.88
	spring 2007	27.77***	11.26*	2.36
	summer 2007	97.83***	24.34**	15.83***
HF (attached)	fall 2006	19.63***	0.62	1.67
	winter 2007	9.48***	1.92	0.28
	spring 2007	6.81***	0.41	0.13
	summer 2007	11.70***	3.98	1.08
Ciliates	fall 2006	244.80***	6.19	3.73**
	winter 2007	73.90***	7.57	2.26
	spring 2007	54.55***	6.77	4.59**
	summer 2007	50.93***	2.27	2.93*
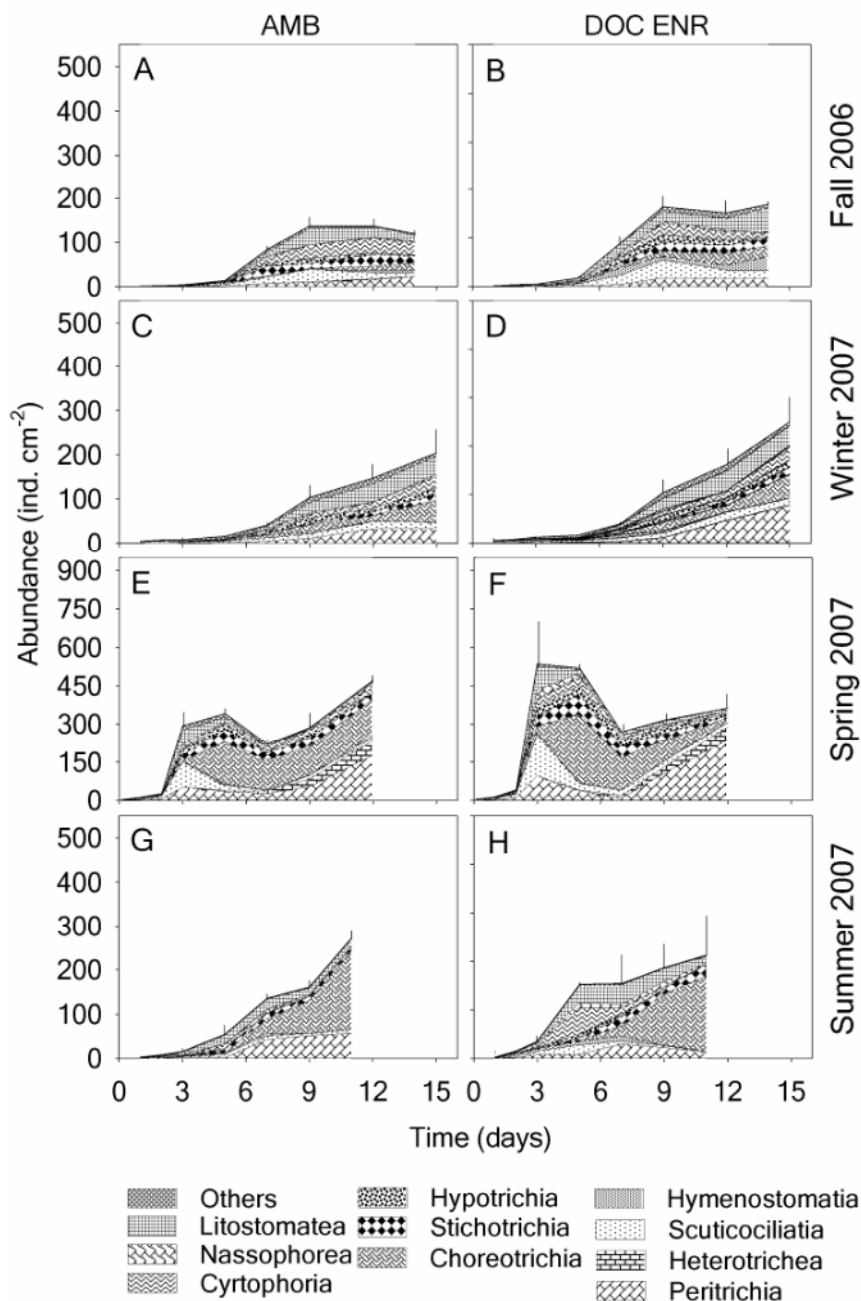
Fig. 4. Influence of stimulated benthic bacterial growth by DOC on the succession of semi-natural ciliate communities over time. The upper bold line in each figure denotes the total abundance (±SD) of ciliates. (A, C, E, G) AMB treatment with no supplemental resource added to the flow cells. (B, D, F, H) DOC ENR treatment with an additional carbon source (yeast extract) fed to the flow cells. Each row represents the ciliate communities of one seasonal experiment as indicated in the figure.

Structural responses of ciliate communities to enhanced resource availability

Figures 4 (AMB vs. DOC ENR) and 5 (BAC CON vs. BAC ENR) show both the numerical and taxonomical succession of the experimental biofilm-dwelling ciliate communities. Both experimental resource enrichments, DOC ENR and BAC ENR, did not result in a seasonal coherent stimulation of specific taxonomic units (either classes or sub-classes). As applied for the total abundances of ciliates and HF, we also performed rmANOVA for the taxonomic succession. The results for this rmANOVA design are given in Table 3.

The observed responses of the experimental ciliate communities to enhanced benthic bacterial growth caused by the DOC ENR were generally slight. However, distinct overall effects of the experimental enrichment appeared as a significant stimulation of hymenostomes in fall 2006 and winter 2007 (Table 3, Fig. 4 A-D). Further significant effects were observed with some surface-grazing taxa: scuticociliates apparently benefit from the resource enhancement as observed for significant ´treatment´ effects in winter and spring 2007 (Table 3, Fig. 4 C-F). Strong effects were also observed for cyrtophorids, which were increased in the DOC ENR in summer 2007. This increase, however, was mainly restricted to the early phase of succession as determined by a significant interaction of ´treatment x time´ ($p<0.01$) in the rmANOVA (Table 3).
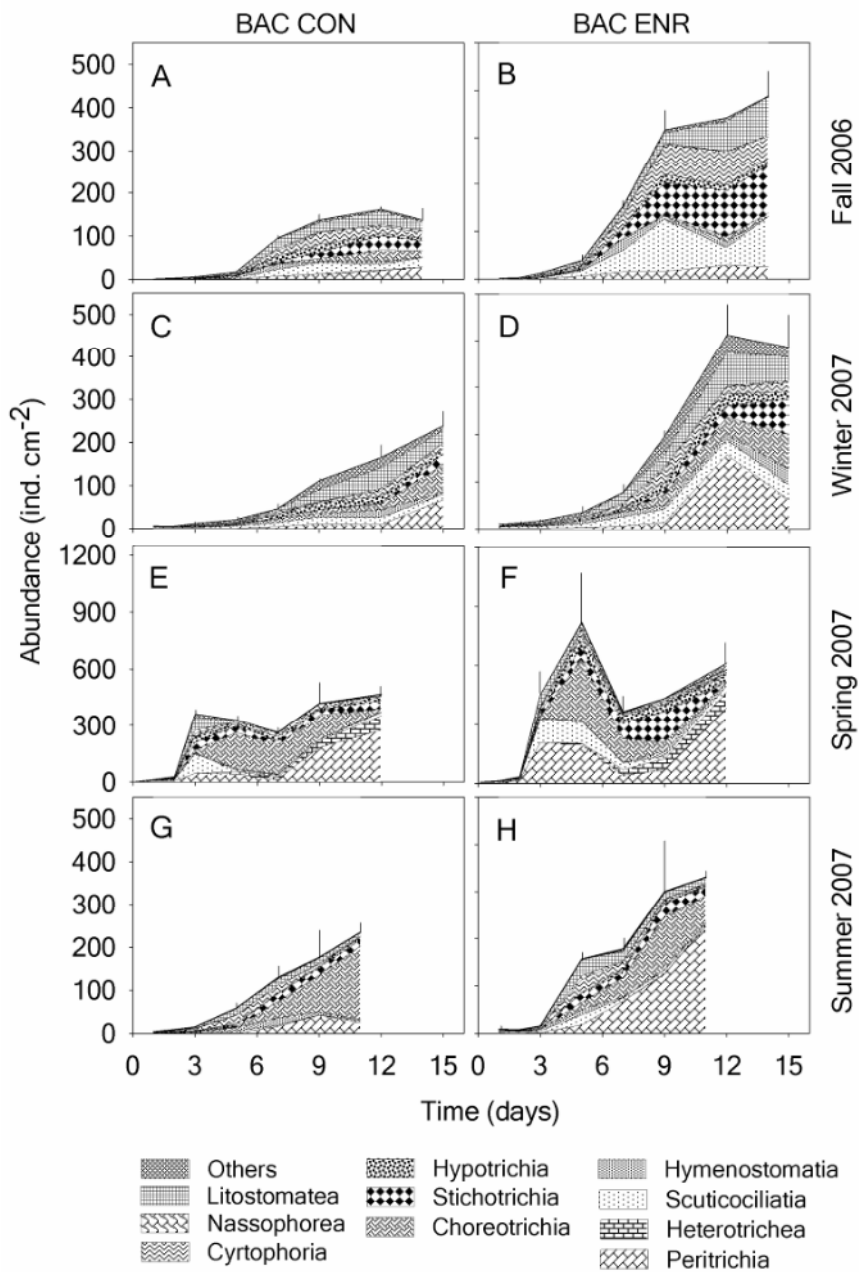
Fig. 5. Influence of enhanced planktonic bacterial density on the succession of semi-natural ciliate communities over time. (A, C, E and G) The BAC CON treatment served as the control treatment for (B, D, F, and H) the BAC ENR treatment which obtained a suspension of bacteria as an additional resource. The upper bold line in each figure denotes the total abundance (±SD) of ciliates. Each row represents the ciliate communities of one seasonal experiment as indicated in the figure.

The influences of the BAC ENR were generally more diverse and pronounced than those of the DOC ENR. As expected from the experimental setup, suspension-feeding ciliate taxa such as scuticociliates and peritrichs were stimulated in all seasonal experiments (for details see Table 3). The significant increase of scuticociliates in fall 2006 was accompanied by a stimulation of litostomes, which was also significant for the interaction ´time x treatment´ ($p < 0.01$). Choreotrichs were negatively influenced by the BAC ENR, i.e. they dominated the BAC CON treatment whereas the BAC ENR was dominated by peritrichs. This differential development of choreotrichs in the two treatments developed during the end of the experimental period and is statistically supported by significant ´time x treatment´ interactions (Table 3, $p < 0.05$). In contrast, stichotrichs were positively influenced by the enrichment with planktonic bacteria. This stimulation was confirmed by the rmANOVAs in fall 2006 and winter 2007 (Table 3, Fig. 6 A-D).

Abundance of rotifers on the experimental biofilms

In fall 2006, winter and summer 2007, rotifers appeared in low densities (generally <5 ind. cm^{-2}) and only towards the end of the experiments. The only remarkable amount of rotifers was recorded in spring 2007 (Fig. 6) with maximal abundances of 29.5±7.5 ind. cm^{-2} in the AMB flow cells. In the DOC ENR, rotifer abundance increased continuously, reaching a maximum of 100±45 ind. cm^{-2} at the end of the experiment (day 12). The maximum rotifer abundance in the BAC CON baseline was 40±0 ind. cm^{-2}; in the BAC ENR treatment it was slightly higher (50±10 ind. cm^{-2}).

Continued from page 73

		Time	Treatment	Time x Treatment
		F (1, 4)	F (7, 28)	F (7, 28)
BAC CON vs. BAC ENR				
Bacteria	fall 2006	-	-	-
	winter 2007	8.50***	1.23	1.35
	spring 2007	1.66	1.63	0.19
	summer 2007	4.81*	3.39	3.81*
HF (vagile)	fall 2006	2.36	5.46	2.75
	winter 2007	19.50***	9.08*	0.72
	spring 2007	22.42***	0.36	1.96
	summer 2007	42.23***	1.36	1.62
HF (attached)	fall 2006	71.04**	20.45*	28.64***
	winter 2007	29.72**	22.71**	20.87***
	spring 2007	7.56	16.63*	6.59***
	summer 2007	45.85**	13.53**	3.37**
Ciliates	fall 2006	158.40***	71.04**	28.64***
	winter 2007	173.71***	29.72**	20.87***
	spring 2007	55.76***	7.56	6.59***
	summer 2007	57.09***	45.85**	3.37**
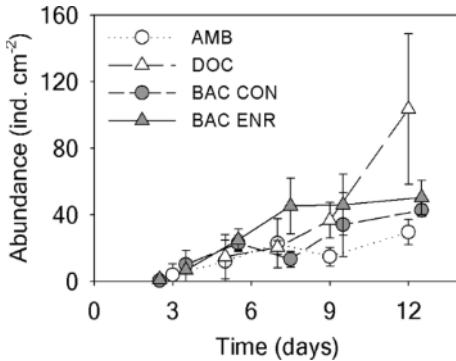
Fig. 5. Abundance of rotifers in spring 2007 on a real-time axis. The symbols for the DOC ENR and the BAC ENR experiments were separated by 0.5 days on the X-axis for optical purposes, even though they refer to the same sample dates. Error bars represent SD. Rotifers only occurred in very low abundances during the other times.

Discussion

The impacts of direct and indirect resource enrichments on biofilm-dwelling ciliate communities were tested for the first time in open river bypass systems. These systems allowed both the direct, controlled manipulation of the resource level as well as live observation of the developing ciliate communities. The high temporal resolution of the experiments facilitated the identification of short-term effects of the enhanced resource availability, which could have been overlooked on a coarser time scale. This problem of missing key events in the development of communities was recognized in different field studies with nutrient enrichments, as discussed by Wickham *et al*. (2004). By combining two different experimental approaches (distinct manipulation of the resource level and type as well as usage of natural ciliate assemblages), we succeeded in detecting seasonal dependencies of the same resource treatments on developing biofilm communities. Both resource enrichments, DOC ENR and BAC ENR, were found to significantly affect the speed with which ciliates colonize biofilms, to influence the ciliate population size and to alter the community composition. However, the magnitude of responses of the

Table 3. Results of repeated measurement ANOVA (rmANOVA) design to test for significant effects of resource manipulations on specific taxonomic units among ciliates in pair-wise comparison (AMB vs. DOC ENR and BAC CON vs. BAC ENR). Values represent the F-ratios and *asterisks* indicate significance (* p<0.05, ** p<0.01, *** p<0.001). Although all taxonomic units (either class or subclass) were tested, the table is limited to those which showed at least one significant between-subjects effect, i.e. either treatment or time x treatment.

Season	Tax. Unit	Time	Treatment	Time x Treatment
AMB vs. DOC ENR		F (5, 20)	F (1, 4)	F (5, 20)
Fall 2006	Hymenostomatia	3.47*	31.47**	2.61
	Nassophorea	1.12	0.36	9.36*
Winter 2007	Hymenostomatia	4.28**	20.29*	3.05*
	Scuticociliatia	5.12**	10.41*	0.94
Spring 2007	Choreotrichia	46.77***	0.20	10.61***
	Scuticociliatia	38.16***	8.10*	2.28
Summer 2007	Cyrtophoria	13.54***	7.52	7.25**
	Hypotrichia	2.94*	1.80	3.30*
BAC CON vs. BAC ENR				
Fall 2006	Litostomatea	12.20***	2.47	5.25**
	Scuticociliatia	11.38***	12.79*	4.94**
	Stichotrichia	7.67***	2.50*	3.44
Winter 2007	Cyrtophoria	16.00***	138.10***	8.69***
	Peritrichia	11.47***	5.36	7.96***
	Scuticociliatia	11.44***	31.30**	1.67
	Stichotrichia	23.86***	9.99*	5.93**
Spring 2007	Choreotrichia	83.03***	0.05	9.81***
	Hymenostomatia	1.52	25.69**	2.47
	Hypotrichia	3.24*	13.09*	3.03
	Litostomatea	31.12***	12.56*	1.10
	Nassophorea	6.08**	1.61	4.97**
	Peritrichia	13.16***	3.79	2.79*
Summer 2007	Choreotrichia	10.74***	0.31	2.74*
	Nassophorea	2.47	4.06	3.16*
	Peritrichia	40.08***	61.42**	23.75***
	Scuticociliatia	6.25**	4.50	3.09*

experimental ciliate communities to resource enrichment strongly depended on both the season as well as on the stage of succession, as shown by different fertilization experiments using field communities (e.g. Andrushchyshyn *et al.* 2006, Sekar *et al.* 2002).

Limited impact of moderate DOC enrichment on biofilm-dwelling ciliate communities

With the addition of supplemental DOC to the developing microbial communities we intended to stimulate the growth of benthic bacteria in order to investigate the trophic linkage between bacterial biofilms and protozoans. This is a controversial subject in the field of microbial ecology, as bacterial biofilms are generally thought of as being resistant to protozoan grazing. This resistance shall result from the quorum-sensing regulated formation of microcolonies and bacterial filaments which can be stimulated by protozoan grazing and which make bacteria further inedible for protozoans (Matz *et al.* 2004; Matz and Kjelleberg 2005). Although recent studies have shown that different stages of bacterial biofilms can be grazed upon by distinct protozoa in the lab (Huws *et al.* 2005; Queck *et al.* 2006; Weitere *et al.* 2005), there has thus far been no evidence for the trophic relevance of bacterial biofilms for protozoan field communities.

We were able to detect manifold effects of the experimental resource enrichment on the developing biofilm-dwelling protozoan communities. In the first instance, a temporary significant stimulation of mostly benthivorous vagile HF was observed in all seasonal experiments. This stimulation has likely resulted from an enhanced utilization of benthic bacteria. However, vagile HF abundance generally declined over the further course of the experiments. There are several possible explanations for this: First of all, the initial HF grazing on the early bacterial biofilms could have induced the formation of inedible bacterial colonies (as demonstrated here by the formation of bacterial filaments) which prevented HF from further grazing upon benthic bacteria and thus resulting in a lack of resources for the vagile HF (as shown for the

flagellate *Rhynchomonas nasuta* when grazing on *Pseudomonas aeruginosa* biofilms in the lab; Matz *et al.* 2004). Furthermore, vagile HF could have been grazed upon by other protists such as ciliates, as we expected when planning the experiments. By this means, particularly cyrtophorids are likely to be responsible for the decline of vagile HF in summer 2007. This interaction will be mentioned later in the discussion.

The next observed effects of the DOC ENR were found for the ciliates. The impact of the DOC ENR on the total ciliate abundance was generally low; the only significant effect appeared as an accelerated speed of colonization of the experimental biofilms in summer 2007. This finding agreed with previous studies, which have shown that DOC enrichment can significantly accelerate initial biofilm colonization by ciliates, especially at high temperatures (Norf *et al.* 2007). More complex responses to the resource enrichment were found with regards to the taxonomic structure of the experimental ciliate communities: Especially surface-browsing ciliates such as cyrtophorids, nassophores, hymenostomes and members of the scuticociliates (e.g. *Cinetochilum*) were significantly stimulated in particular seasonal experiments. It is very likely that the observed significant increase of ciliate taxa such as cyrtophorids and nassophores was a response to the enhanced HF abundance (see above) rather than to an enhanced abundance of biofilm bacteria (Franco *et al.* 1998). This assumption was confirmed by live observations of these ciliates grazing on vagile HF. Especially cyrtophorids have accounted for the significant enhanced colonization speed in summer 2007 (Figs. 2, 4). In contrast, the significant stimulations of hymenostomes and scuticociliates could have been due to the stimulated benthic bacterial growth itself, as members of both taxa are known to be capable of grazing efficiently on either suspended or attached bacteria, as shown for interstitial habitats (Königs and Cleven 2007). However, members of these two taxa exhibited different succession patterns within the experiments. The scuticociliate *Cinetochilum*, for instance, was mainly present in the earliest phases of succession and presumably grazed on loose bacteria and small bacterial colonies. In contrast, larger amounts of hymenostomes appeared as

soon as bacterial filaments had developed within the flow cells in fall 2006 (data not shown) and winter 2007 (Fig. 1). In this experiment, especially the hymenostome *Glaucoma* was observed to glide on the bacterial filaments and remove short fragments from their ends. However, *Glaucoma* was the only ciliate that could have benefited from the formation of otherwise grazing-resistant bacterial morphotypes. In the majority of cases, we could not find indications for the propagation of the bacterial filament biomass to higher trophic levels. These weak responses of bacteriovorous ciliates in our experiments was surprising, as recent lab studies have shown that ciliates from different taxa can efficiently reduce even mature bacterial biofilm biomass (Huws *et al.* 2005; Weitere *et al.* 2005). Nevertheless, the indirect stimulation of ciliates due to the preceded grazing of vagile HF that benefited from the enhanced bacterial growth confirms that HF can act as a trophic link between bacteria and higher trophic levels also within biofilms, as known for the plankton (Azam *et al.* 1983).

The ambient DOC load in the River Rhine at any particular season (Table 1) did not allow any a-priori prediction on how the experimental communities would react to the manipulative input of 0.005 mg l^{-1} yeast extract. This is probably due to the fact that the amount of refractive vs. biologically available DOC is determined by several environmental variables (Meyer 1994). On the other hand, the overall slight response to our experimental supplementation of a defined DOC amount is dose-dependent. It should be noted, however, that a slightly higher dose of supplemental yeast extract (0.01 mg l^{-1}) was already found to cause excessive growth of bacteria within the flow cells in temperature manipulation experiments (Norf *et al.* 2007). This was accompanied by strong changes in the microenvironment (such as oxygen depletion), as indicated by the occurrence of protozoan taxa typical for an environment with oxygen deficiency. The aim of the present paper was to explicitly consider the impact of a moderate DOC supplement on a given grazer community rather than to track drastic changes in response to high DOC loads.

Enhanced planktonic bacterial density can significantly affect ciliate community structure

Compared to the generally weak effects of the DOC ENR on ciliate communities in our experiments, the responses of ciliates to the enhanced availability of planktonic bacteria were distinct and pronounced. Calculation of the factor t_{50} revealed that the density of planktonic resources is a strong trigger for the colonization speed of biofilms by ciliates, as the enrichment of planktonic bacteria sufficed to accelerate initial colonization significantly in all seasonal experiments. This reduction of t_{50} was weakest in spring 2007 - the season with the highest load of planktonic bacteria and algae by far (Table 1) - showing the smallest (but still significant) limitation of planktonic bacteria for the biofilm-dwelling ciliates in this season.

As we expected when planning the experiments, strong responses to the increased planktonic bacterial density occurred for suspension-feeding ciliate taxa such as scuticociliates and peritrichs, which were able to benefit directly from the enhanced resource availability as shown by Posch *et al.* for the scuticociliate *Cylidium* (Posch *et al.* 2001) and Eisenmann *et al.* for the peritrich Vorticella (Eisenmann *et al.* 2001). The response of scuticociliates to the BAC ENR was strongest when the background abundance (as shown in the BAC CON and AMB treatments) of peritrichs was low, as observed in fall 2006. In winter, spring and summer 2007, peritrich abundance was significantly enhanced due to the BAC ENR. However, their enhancement was often accompanied by a reduction in the number of suspension-feeding choreotrichs; this reduction was significant in spring and summer 2007. Taken together, these findings could argue for competitive exclusion of suspension feeding ciliates which is altered by the level of the planktonic resources. The strength depends on the seasonal/environmental factors. These differential effects of the taxonomic groups and of the experimental times show that the effects of plankton enrichment are far more complex than a simple, homogenous stimulation of suspension-feeding ciliates, and are thus difficult to predict.

In addition to the direct utilization of planktonic bacteria, we also observed indirect stimulation of different ciliate taxa in the BAC ENR treatment. The stimulation of collecting taxa such as cyrtophorids, nassophores and hypotrichs was most likely due to an increase in the vagile HF abundance as observed in the DOC ENR experiments (see above). The significant increase of litostome ciliates can possibly be assigned to the dramatic increase of small ciliates such as scuticociliates, which can be preyed upon by litostomes like *Acineria* and *Litonotus*; Parry 2004). The finding of a significant stimulation of stichotrichs, however, was surprising, as most members of this taxon are not capable of grazing on bacteria very efficiently, as demonstrated by Pfister and Arndt (1998). However, these authors found that stichotrichs are able to graze efficiently on larger unicellular organisms such as flagellates or even small ciliates and thus are capable of omnivory. Our observation could possibly account for these experimental findings: The prominent increase in the number of stichotrichs was observed as soon as small ciliates (e.g. scuticociliates) or sessile HF began to establish themselves within the flow cells. The abundances of the latter organisms was greatly reduced when stichotrichs were abundant. However, more specific research is needed to directly track the matter flux pathways and the involvement of the specific groups within biofilms.

Both the significant acceleration of biofilm colonization as well as the numeric enhancement of ciliate taxa due to the planktonic resources were generally continued over the course of the experiments and resulted in maximal effects towards the end of the experiments in fall 2006, winter and summer 2007. Similar stimulations of the ciliates' abundances by increased resource levels have been found in other enrichment experiments (Domenech *et al*. 2006; Ribblett *et al*. 2005, Wickham *et al*. 2004). However, in contrast to the general abundance enhancement, maximum ciliate abundance was observed on day five in the BAC ENR flow cells in spring 2007 before dramatically decreasing by approximately 50% and equilibrating with the flow cells in the BAC CON treatment. A likely explanation for this phenomenon was the occurrence of high

rotifer abundances, which was observed only in this experiment. Rotifers serve as important predators of ciliates as demonstrated in the plankton (Joaquim-Justo *et al*. 2004; Mohr and Adrian 2002). The same phenomenon was observed at the same time in the DOC ENR experiment. The results thus strongly suggest that the stimulation of the ciliate biomass by both planktonic bacteria and DOC was transferred to higher trophic levels, where it results in a stimulation of the rotifer biomass (at least temporarily). This finding is in agreement with studies by Hillebrand *et al* (2002), who found that enhanced protozoan abundance due to previous nutrient enrichments could be directly transferred to grazers, so that an effect on lower trophic levels would become undetectable. The high temporal resolution in our experiments allowed us to track early ciliate responses to resource enrichment before rotifer grazers became abundant, which would have been overlooked on a coarser time scale.

Conclusion: The relevance of resource type and seasonal setting

The availability of resources was shown to be an important factor in shaping biofilm-dwelling microbial communities. Planktonic bacteria were shown to be one important resource for biofilm-dwelling consumers. In contrast, biofilm-dwelling bacteria (though exerting significant effects) were of limited importance. This suggests that biofilm-dwelling consumers act rather as pelagic-benthic couplers than as biofilm-grazers. Reasons for the limited effect of DOC are on the one hand the indirect (and thus less efficient) pathways via vagile HF and on the other hand the formation of grazing-resistant bacterial morphs in the biofilm. The latter point agrees with the general concept of grazing-avoidance strategies in bacteria by the formation of biofilms (Matz and Kjelleberg 2005). Our study is among the first to address the relevance of such issues on complex natural communities and partly supports the hypotheses of a limited carbon transfer from biofilm-bacteria to potential biofilm-consumers. Nevertheless, grazing of biofilms by late-biofilm colonizers such as amoebae

has been observed in laboratory systems (Queck *et al.* 2006; Weitere *et al.* 2005), and our observations demonstrate that a limited utilization of largely grazing-resistant bacterial filaments by ciliates was possible. Thus, further studies are required to test the utilization of bacterial biofilms by protozoans in later succession stages.

Another significant finding of the present study was that the results obtained were not predictable from the ambient resource setting in the River Rhine for any season and both the responses of the different taxonomic groups as well as that of the total abundance varied between the experiments. We suggest that a strong interaction of the resource availability with other environmental variables is likely to be responsible for this finding. For example, it has been shown that experimental temperature increases can accelerate the colonization speed of biofilms by ciliates as long as the resource availability does not constrain their growth (Norf *et al.* 2007). Nevertheless, despite the interactive effects with other factors such as temperature, our results show significant resource effects in any season, at least for BAC ENR. This shows that the ciliate communities were generally resource-limited, as suggested by Scherwass and Arndt (2005) for planktonic ciliate communities in the River Rhine. However, the weakest responses to the experimental resource enrichments on ciliates were observed at low ambient temperature (suggesting that temperature is a more limiting factor than resource availability in colder seasons), whereas ciliate growth at higher temperatures is primarily constrained by the availability of resources.

Chapter 4.

Bottom-up vs. top down control of ciliate communities: Effects of resource supplements on mature biofilms.

Abstract

Three experiments were performed to test the impact of resource supplements on mature biofilm-dwelling ciliate communities using a novel type of flow cells. After eight weeks of pre-cultivation in the River Rhine, biofilms containing ciliate communities were transferred to these flow cells and the resource density was experimentally increased by supplementing the water flow with planktonic bacteria. In two of the experiments, the ciliates were initially stimulated with respects to taxon-specific responses to the bacteria supplement. However, their density decreased over the course of the experiments until no difference to the control treatment (no resource supplement) could be detected. This finding was accompanied by an enhanced abundance of micrometazoans that preyed on the ciliates in the supplemented flow cells. Likewise, no effects of the resource supplement on the ciliate communities were detected when micrometazoans were *ab initio* abundant, as observed in one experiment, despite of a slight adjustment in the structure of the ciliate communities due to the partial replacement of smaller ciliates by larger ones. Taken together, the results show that mature ciliate communities are strongly influenced by top-down mechanisms (grazer activity) rather than by bottom-up factors (the resource availability). Although increased resource densities can temporarily stimulate ciliate communities, this also enhances the grazers of the ciliates so that no resource effect on the standing crop of the ciliate communities becomes obvious.

Introduction

Microbial assemblages on surfaces play an important role in the matter flux of aquatic ecosystems, especially in shallow- and running waters (Bryers and Characklis 1982; Fischer *et al.* 2002). These so-called biofilms (Wetzel 2001) are composed from variety of prokaryotic and eukaryotic organisms among which ciliates oftentimes exceed the biomass of the microbial community (Finlay and Esteban 1998; Gong *et al.* 2005). Ciliate communities consist of a variety of taxa that differently contribute to nutrient cycling processes (reviewed in Arndt *et al.* 2003; Parry 2004). Such differences arise from a high functional diversity which is remarkably higher for ciliates than for other biofilm-dwelling protozoans (Finlay and Esteban 1998). Depending on the specific feeding mode, ciliates can ingest resources of different particle size and from different origin including both benthic and planktonic resources (Finlay and Esteban 1998; Parry 2004). Being one of the most fundamental parameters for both population and community growth rates (Thouvenot *et al.* 2003), the resource density can determine both the carrying capacity and the structure of ciliate communities as reported in both field surveys on planktonic ciliate communities (Wiackowski *et al.* 2001; Andrushchyshyn *et al.* 2003; Scherwass and Arndt 2005; Tirok and Gaedke 2007) and in manipulative studies with resource enrichments (Diehl and Feissel 2000; Wilcox *et al.* 2005; Andrushchyshyn *et al.* 2006).

Field studies have demonstrated that biofilm-dwelling ciliate communities, too, can significantly respond to experimental resource supplements (Hillebrand *et al.* 2002; Wickham *et al.* 2004; Domenech *et al.* 2006; Andrushchyshyn *et al.* 2006). Such studies were generally performed by the indirect manipulation of the resource level via producers (stimulated by, e.g., fertilizers). Yet, little is known of the distinct impacts of controlled (direct) resource supplements on the ciliate community structure. Furthermore, the studies were mainly focused on investigating sum responses of the ciliate communities before and after resource enrichments, rather than on resource induced (short-term) dynamics. Due to the high growth rates of ciliates (Müller and Geller 1993), it is likely that

mature ciliate communities (holding high organism densities) could respond rapidly to changes in the resource density. However, investigating both the distinct impacts of resources as well as short-term responses resource supplements with natural biofilm-dwelling ciliate communities is constrained by the poor experimental accessibility of biofilms in the field. This imposes a challenge for the development of appropriate experimental facilities.

One possible approach for testing the impacts of particular factors on biofilm development was presented in Norf *et al.* (2007) where ciliate communities were cultivated in miniature flow cell systems used as river bypass systems. The flow cells thereby facilitate both the controlled manipulation and the continuous observation of developing biofilm-dwelling ciliate communities. In further experiments, the flow cells were used for direct adjustment of the resource level by the addition of particular resources to the water flow. In doing so, increased planktonic bacteria density was shown to significantly affect the early development of biofilm-dwelling ciliate communities, starting from blank surfaces (see Chapter 3). Despite of a generally high sensitivity of the ciliate communities to increased bacterial density, these experiments further demonstrated the importance of the environmental background, which can strongly influence the magnitude of community responses. Important factors were the ambient resource load (planktonic bacterial density) and the activity of micrometazoan grazers that were found to prey on the ciliates immediately after their enhancement in one experiment.

While the previous experiments concentrated on investigating the effects of bacteria supplements on the early development of biofilm-dwelling ciliate communities (the biofilm succession started from a sterile flow cell surface), they do not provide specific information about the possible responses of mature ciliate communities. In contrast to early biofilm-dwelling ciliate communities (two to four weeks old), mature (>8 weeks) communities mainly consists of attached and colonial ciliates (Primc-Habdija *et al.* 2005; Gong *et al.* 2005; Mieczan 2005) that can effectively graze on suspended prey (Eisenmann *et al.* 2001). Due to the high growth rates of ciliates (Müller and Geller 1993), mature

ciliate communities thus could strongly benefit from enhanced bacterial densities. This could enhance their productivity and increase the carrying capacity of the communities. Furthermore, mature biofilms exhibit a higher density of micrometazoans, which can exert stronger grazing pressures on ciliates compared to early biofilms. Hence, it is possible that resource supplements could enhance the grazer activity so that no effect on the ciliate community will become obvious. However, Epstein and Gallagher (1992) found no correlation between meio- and macrofauna abundance and benthic ciliate density indicating that the ciliate density could primarily depend on the availability of resources rather than on the activity of grazers.

In order to investigate the impact of temporary increased planktonic bacterial densities on the dynamics of mature biofilm-dwelling ciliate communities, a novel type of flow cells was invented which facilitates the controlled manipulation of naturally pre-cultivated biofilms. The biofilms for the experiments were cultivated on object slides which were exposed in the River Rhine for eight weeks in order to achieve ciliate communities at carrying capacity. After transferring the biofilms to the new flow cells, they were fed with a planktonic bacterial suspension besides of the river water bypass to untreated river water. Acknowledging that both bottom-up (resource densities) and top-down (consumers of the ciliates) factors can be involved in controlling ciliate community structure, we tested the impact of the resource supplement on micrometazoans as well. In doing so, the following questions were addressed: (1) Do mature ciliate communities respond to resource supplements and how quickly do such responses appear? (2) Do resource supplements also influence potential consumers of the ciliates (micrometazoans), thus altering the interaction strength between ciliates and micrometazoans? (3) Can enhanced resource densities sustainalbly influence the ciliate communities and increase the standing stock (carrying capacity) of the communities?

Material and Methods

Experimental design and culturing methods

Three experiments (March, May and August 2007) were performed to test the responses of mature biofilm-dwelling ciliate communities to increased food densities by experimentally increasing the density of planktonic bacteria which was shown before to significantly influence early biofilm-dwelling ciliate community dynamics (see Chapter 3). The ciliate communities were pre-cultivated on object slides which were exposed for eight weeks in flumes fed with untreated Rhine River water (0.2 m s^{-1}) aboard the Ecological Rhine Station (University of Cologne). Afterwards, object slide each was transferred to a novel type of flow-cell (Fig. 1) which is suitable for both the direct manipulation and the live observation of the same pre-grown biofilms over an extended time-span. The first community analyses, providing the base line for the experiments (day zero), were performed after an acclimatization period of 24h of the biofilms after transferring to the flow cells (see below).

The flow cells principally work like those utilized in earlier experiments, where ciliate communities were cultivated starting from blank surfaces (Norf *et al.* 2007; Wey *et al.* submitted). They consist of a plastic frame with a dimension of 80 x 35 cm^2 and an internal 15 ml volume in the space between the upper object slide and the base of the flow cell. The plastic frame holds three miniature pipes on two sides of the flow cells each which serve as the inflows and the outflows for the Rhine River water bypass.
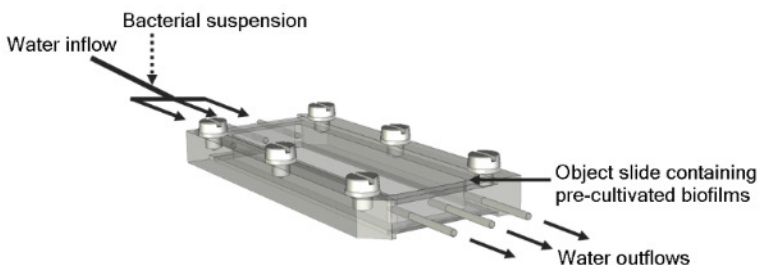
Fig. 1. Flow cell as used for manipulation and monitoring of mature biofilm-dwelling consumer communities. For the experiments, biofilms were pre-grown on object slides and adjacently transferred to the flow cells.

A combined sediment and bubble trap was installed in front of the inflows in order to reduce the amount of fine-grained sediment from the water flow and to keep otherwise destructive air bubbles from the biofilms. The flow cells (including the mature biofilms) were attached to a permanent bypass of untreated river water via tube pumps (one volume exchange min^{-1}). Four of the flow cells were maintained with no further treatment giving the CONTROL setup (no resource added) which only obtained the suspended resources from the water flow. Four additional flow cells were additionally fed with a suspension of non-toxic planktonic bacteria (*Pseudomonas putida* MM1; (Dütz *et al.* 1994) besides of the river water bypass giving the BACTERIA SUPPLEMENT treatment. Previous experiments using a similar setup have shown that the addition of *P. putida* to biofilms can significantly stimulate ciliate communities (see Chapter 3). For preparation of the bacterial suspension, kryo-preserved *P. putida* were cultured in Erlenmeyer flasks containing 100 ml 50% M9 culture medium (Hahm *et al.* 1994) +0.04 g l^{-1} glucose at room temperature (20°C). The cultures were harvested by centrifugation (3,400 g; 15 min.) after two days of cultivation. This was shown to reduce the ability of biofilm formation with *P. putida* (Bell *et al.* 2005). The obtained pellet was resuspended in Pratt minimal medium (Pratt and Salomon 1980) in order to wash the residual glucose from the cultures. The bacteria were then harvested again as described above.

Table 1. Ambient setting of selected environmental variables during the experiments. Water temperature was logged daily. Planktonic bacteria (DAPI countings) and algae (live countings) abundances were determined at the beginning and at the end of the experiments. The given values are the mean values (±SD) for all replicates of the two observations.

Experiment	Date	Temp ($^{\circ}$C)	Bacteria (10^6 cells ml^{-1})	Algae (cells ml^{-1})
Mar 2007	March 10.-22. 2007	9.4±0.6	1.6±0.2	718±176
May 2007	May 7.-19. 2007	17.5±0.7	2.6±0.6	1750±232
Aug 2007	August 8.-22. 2007	19.8±0.7	1.6±0.3	417±111

After the cell density was determined by the use of a Helber counting chamber (W. Schreck, Hofheim, Germany), a suspension containing 1×10^8 bacteria ml^{-1} (final concentration) was prepared in standard reagent bottles. This suspension was then fed to the flow cells with sterile silicone hoses via tube pumps. The bacteria solution was diluted 1:100 with Rhine River water in the flow cells so that the final concentration of *P. putida* in the flow cells was 10^6 ind. ml^{-1}. This concentration of bacteria was shown to strongly increase the abundance of biofilm-dwelling ciliates within early biofilms (Chapter 3). Data on the ambient density of planktonic bacteria in the River Rhine for the experiments is given in Table 1. The bacteria suspension was stored in a water bath ($6^{\circ}C$) during the experiments and was renewed every two days.

Data analysis

The biofilm-dwelling ciliate communities were analyzed microscopically by placing the flow cells directly under the microscope. The first survey was performed after an acclimatization period of 24 hours and before the resource supplement was applied (day zero), providing a baseline for the further observations. A minimum of 60 ciliates pre flow cell were counted in defined areas which were randomly distributed over the biofilms at 100x magnification at each survey. Due to the high density of colonial ciliates on mature biofilms, their abundance was optically recorded separately in a larger area at 50x magnification. Ciliate identification was performed according to the identification keys of Foissner & Berger (1996) and Lynn and Small (2002). In addition to the ciliate communities, micrometazoan abundances were recorded. They were either determined in the same areas as the colonial ciliates (applied for gastrotrichs, insects, rotifers) or for the complete biofilm area (applied for oligochaetes and platyhelminths). After the first community analyses on day zero, the biofilms were randomly assigned to the experimental treatments (AMB; BAC). The designated BAC flow cells were additionally attached to the prepared bacteria suspensions. The following community analyses were started one day after the initiation of the resource supplement. The statistical analysis

concentrated on investigating the effects of increased bacterial density on the biofilm-dwelling ciliate communities as well as on micrometazoans. Therefore, repeated-measure ANOVAs (hereafter: rmANOVA) were calculated with <u>time</u> as the inner-subject factor and <u>treatment</u> (AMB vs. BAC) as the between-subject factor. In doing so, the effects of increased resource density on the total abundance of ciliates as well as on the abundance of specific taxonomic units (classes or sub-subclasses for ciliates and classes for micrometazoans) as dependend variables were analysed. Concerning the micrometazoans, the rmANOVA were performed for likely micrometazoan grazers of ciliates (oligochaetes, platyhelminths and rotifers) besides of the analyses of effects on the total abundance of micrometazoans.

Results

### Starting conditions of the experiments

The biofilm-dwelling consumer communities exhibited similar starting conditions within the experiments; however, particular seasonal differences between the experiments occurred. In March and August 2007, starting ciliate abundances were rather similar. The highest ciliate abundance was recorded in May 2007 being the experiment with the highest ambient resource load (Table 1). Regarding the ciliate community structure, peritrichs were initially dominant among the biofilms and generally accounted for >60% of the total ciliate population. Similar to the ciliates, micrometazoan density was highest in May 2007 (>30 ind. cm^{-2}) and comparably low in March and August 2007 (<10 ind. cm^{-2}).

Effects of bacteria supplements on biofilm-dwelling ciliate dynamics

In March 2007, the ciliate abundances in the AMB flow cells ranged between 290±50 (day zero) and 530±140 ind. cm^{-2} at the end of the experiment (day 12) due to an increase of the ciliate abundance within the first days of the experiment (Fig. 2a). Concerning the taxonomic structure, peritrichs were

generally dominant with exception of day zero, where choreotrichs contributed to ca. 20% of the communities. However, the choreotrichs were replaced by peritrichs after one further day of cultivation. Litostomes and scuticociliates were generally low represented (<10%). The BACTERIA SUPPLEMENT resulted in a rapid increase in the ciliate abundance from 320 ± 40 ind. cm^{-2} to a maximum of 1050 ± 360 ind. cm^{-2} on day six before decreasing to a final abundance of 440 ± 40 ind. cm^{-2} being not different from the ciliate abundance in the AMB (Fig. 2a). Calculations of the rmANOVA reported significant effects of the BAC treatment on both the total ciliate abundance and on specific ciliates (Fig. 2b, c; Table 2). The effects were strongest for peritrichs as reflected by significant effects in the factors <u>time</u> (p<0.001) and <u>treatment</u> (p<0.05) as well as by significant interactions <u>time x treatment</u> (p<0.001). The second significant effect was an increase of litostomes in the flow cells which obtained the BAC treatment.
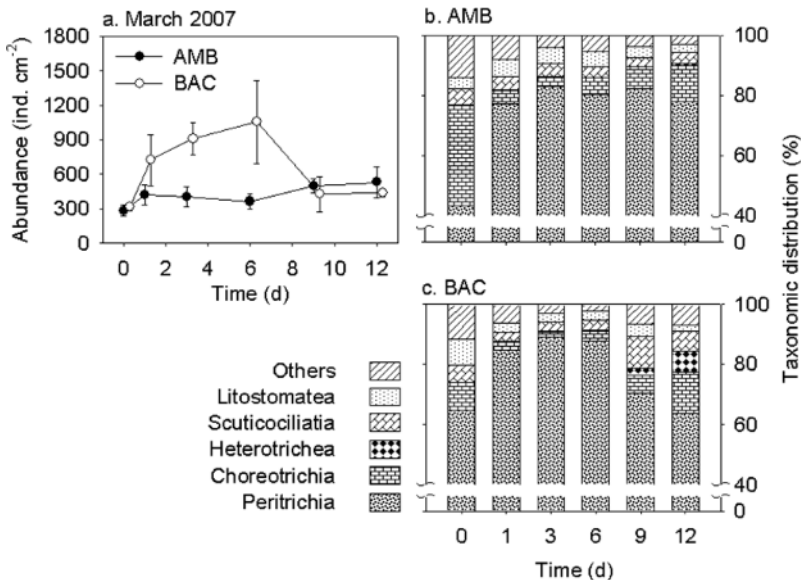
Fig. 2. Response of biofilm-dwelling ciliates to bacteria supplement in March 2007. (a) Total abundance of ciliates on a real-time axis. (b, c) Taxonomic contribution of selected taxa to the total ciliate abundance in the flow cells without (b) and with bacteria supplement (c). Note the axis break in the panels b and c.

In May 2007, different results were obtained. The ciliate abundances in both treatments were rather similar throughout the experimental period and there was no significant effect of BAC detected on the ciliates. Significant group effects were detected on choreotrichs, which increased in the BAC treatment until day three (Fig. 3b; Table 2) as reported by a significant time effect (p<0.001) in the rmANOVA.

Another effect appeared among large heterotrich ciliates, which were significantly stimulated towards the end of the experiment in the BAC treatment (Fig. 3c; Table 2.) as reflected by a significant time effect (p<0.001) as well as by significant interactions time x treatment (p<0.001).
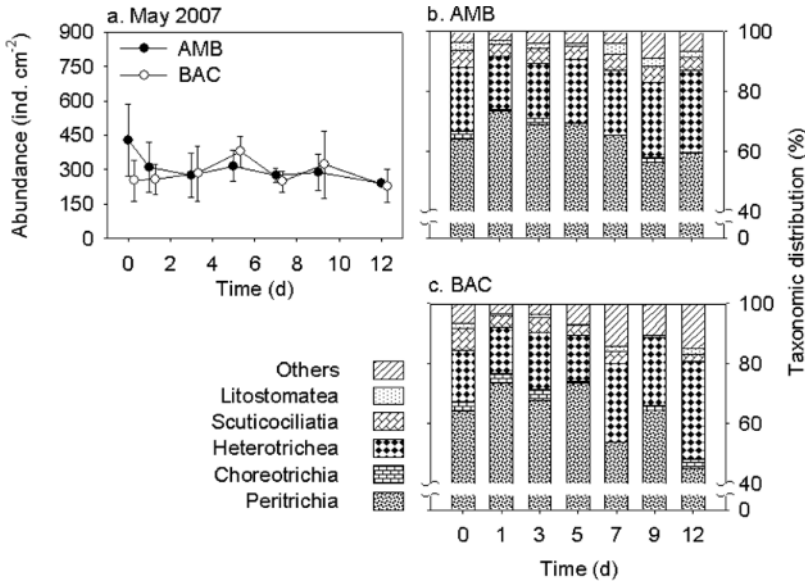
Fig. 3. Response of biofilm-dwelling ciliates to bacteria supplement in May 2007. (a) Total abundance of ciliates on a real-time axis. (b, c) Taxonomic contribution of selected taxa to the total ciliate abundance in the flow cells without (b) and with bacteria supplement (c). Note the axis break in the panels b and c.

In August 2007, the ciliate abundance was initially higher in the AMB (530 ± 120 ind. cm^{-2}; Fig. 4a) than in the BAC flow cells (440 ± 120 ind. cm^{-2}; Fig. 4a). After a short increase within the first day of the experiment, ciliate abundance remained constant (ca. 800 ind. cm^{-2}) and increased to a maximum of 1170 ± 400 ind. cm^{-2} on day five in the BAC treatment before equilibrating in both treatments at the end of the experiment (Fig. 4a). The rmANOVA revealed multiple significant responses on the ciliate communities to the BAC supplement (Table 2): Besides of a strong significant impacts on the total ciliate abundance on the factors time ($p<0.05$) and treatment ($p<0.001$) as well as in the interaction time x treatment ($p<0.05$), significant effects were detected for heterotrich, litostome, and peritrich ciliates and for scuticociliates (see Table 2 for details).
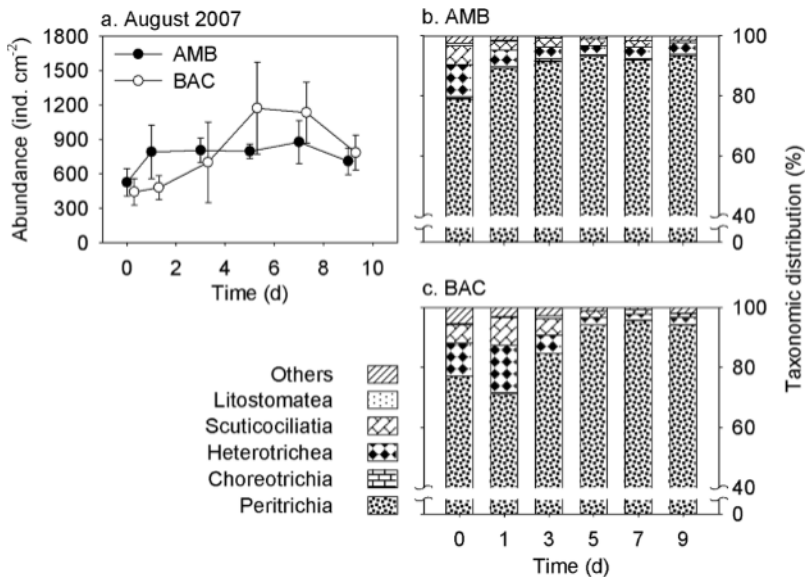
Fig. 4. Response of biofilm-dwelling ciliates to bacteria supplement in August 2007. (a) Total abundance of ciliates on a real-time axis. (b, c) Taxonomic contribution of selected taxa to the total ciliate abundance in the flow cells without (b) and with bacteria supplement (c). Note the axis break in the panels b and c.

Table 2. Results from the repeated-measures ANOVAs testing the effects of bacteria supplement on biofilm-dwelling ciliate communities. The rmANOVA were calculated for the total abundance of ciliates and for the abundant taxonomic groups as indicated in the corresponding figures 2-4. (Chor) Choreotrichia, (Het) Heterotrichia, (Lit) Litostomatea, (Per) Peritrichia, (Scut) Scuticociliatia. **Bold values** indicate significance.

		Time			Treatment			Time x Treatment		
		ss	F	p	ss	F	p	ss	F	p
March 2007	Ciliates	647,657	6.18	**0.001**	468,520	15.88	**0.016**	789,053	7.53	**>0.001**
df_{time} (5, 20)	Chor	13,465	2.80	**0.045**	900	0.56	0.496	6,025	1.25	0.323
$df_{treatment}$ (1, 4)	Het	1,166	2.64	0.055	491	2.73	0.174	874	1.98	0.126
$df_{time \times treatm}$ (5, 20)	Lit	855	4.15	**0.010**	187	0.67	0.460	574	2.79	**0.046**
	Per	808,488	8.68	**>0.001**	380,009	13.12	**0.022**	737,163	7.91	**>0.001**
	Scut	599	1.06	0.414	842	1.48	0.240	1,481	4.08	0.114
May 2007	Ciliates	59,255	2.23	0.075	5,783	0.18	0.692	52,406	1.97	0.110
df_{time} (6, 24)	Chor	537	4.35	**0.004**	0.05	0.00	0.978	68	0.55	0.767
$df_{treatment}$ (1, 4)	Het	6,598	24.18	**>0.001**	144	0.81	0.418	2,090	7.66	**>0.001**
$df_{time \times treatm}$ (6, 24)	Lit	162	1.01	0.444	136	1.91	0.239	30	0.19	0.977
	Per	25,718	0.70	0.650	4,293	0.10	0.766	54,676	1.49	0.222
	Scut	961	2.10	0.091	434	1.64	0.270	365	0.80	0.581
August 2007	Ciliates	1,213,108	6.62	**0.001**	21,247,094	250.73	**>0.001**	481,183	2.63	**0.045**
df_{time} (5, 20)	Chor	29	0.28	0.918	151	12.57	**0.024**	45	0.44	0.815
$df_{treatment}$ (1, 4)	Het	1,554	0.98	0.457	20,	0.12	0.743	578	0.37	0.893
$df_{time \times treatm}$ (5, 20)	Lit	63	3.19	**0.028**	0.962	0.09	0.783	82	4.21	**0.009**
	Per	1,468,975	7.73	**>0.001**	4,484	0.05	0.833	553,889	2.91	**0.039**
	Scut	3,184	5.30	**0.003**	273	1.04	0.366	707	1.18	0.355

Effects of bacteria supplements on micrometazoans

Like observed for the ciliate communities, the micrometazoan communities, too, exhibited strong variations with regards to the base conditions (day zero) and to the resource manipulation. The large differences in the total micrometazoan abundances between the experiments (Fig. 5a-f) were accompanied by strong variations in the composition of the communities. The following description will concentrate on the effects of the resource supplement on micrometazoans which could have benefited from increased ciliate abundances due to their reported ability to graze on ciliates. Thus oligochaetes, platyhelminths and rotifers were included in the statistical analyses (Table 3).

In March 2007, the total micrometazoan density was low and mainly constituted by rotifers in both the AMB and the BAC flow cells. In the AMB, rotifer abundance ranged between 3.5 ± 3.5 and 4.9 ± 6.2 ind. cm^{-2}. Similar abundances were observed in the BAC treatment with 5.0 ± 4.9 ind. cm^{-2} rotifers. Unlike the AMB treatment, supplementation of BAC induced a strong increase towards day six (11.5 ± 6.6 ind. cm^{-2}) as reported by a significant <u>treatment</u> effect in the rmANOVA (Table 3) before decreasing to 4.9 ± 2.0 ind. cm^{-2}. *Ab initio* higher micrometazoan densities compared to the other experiments were observed in May 2007 (Fig. 5c, d) with 49.5 ± 10.7 ind cm^{-2} in the CONTROL and 31.7 ± 10.7 ind. cm^{-2} in the BAC flow cells on day zero. After starting the experiments, micrometazoans decreased to 27.7 ± 4.3 ind. cm^{-2} (AMB) and 20.8 ± 4.3 ind. cm^{-2} (BAC). Significant responses to the BAC were detected for the total micrometazoan abundance (Table 3; time, $p < 0.05$), in particular for rotifers (<u>time</u>, $p < 0.05$; <u>time x treatment</u>, $p < 0.05$), which were temporarily increased on day three and for platyhelminths (<u>time</u>, $p < 0.05$; <u>time x treatment</u>, $p < 0.05$) which responded positively to the supplement on day seven and nine.
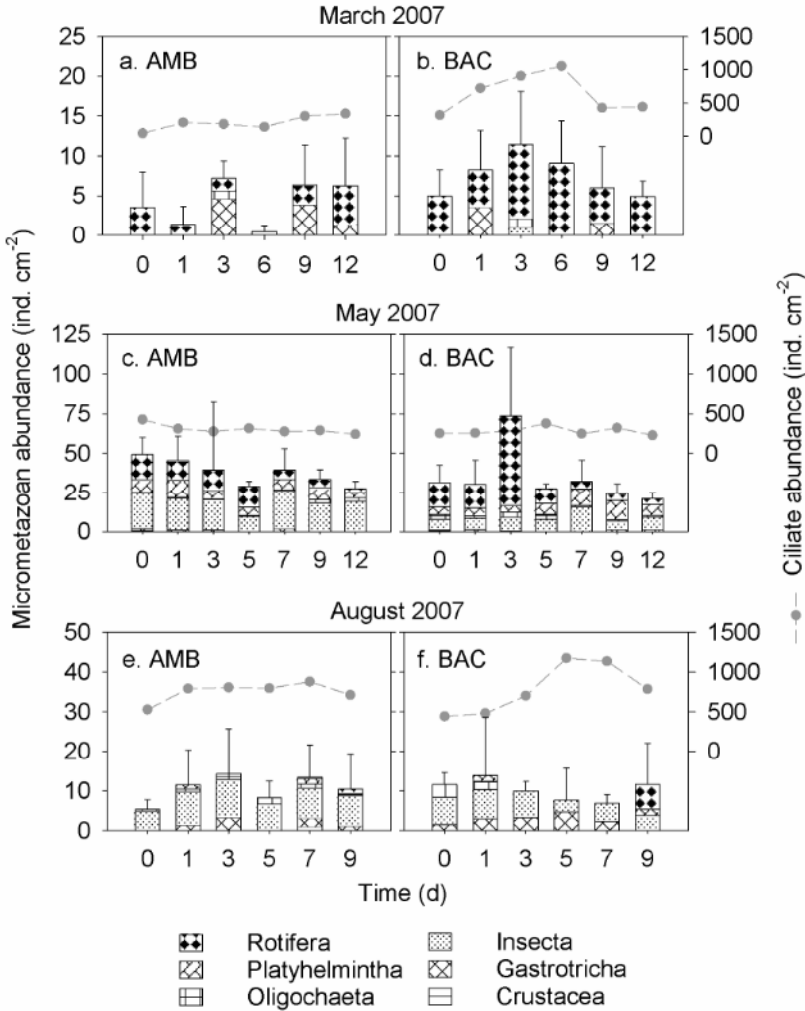
Fig. 5. Impact of bacteria supplement on biofilm-dwelling micrometazoans. Figures a-f give a combined illustration of the total abundance and the taxonomic composition of the micrometazoan communities in the flow cells without (left column) and with (right column) bacteria supplement. (a, b) March 2007. (c, d) May 2007. (e, f) August 2007. Ciliate mean abundances (dots and lines) were included in the figure for comparison (see Figs 2-4). Error bars (±SD) refer to the total abundance.

Table 3. Results from the repeated-measures ANOVAs testing the effects of bacteria supplement on micrometazoans. The analysis were concentrated on those grazers which were likely consumers of the ciliates. In March 2007, platyhelminths were not present. Bold values indicate significance.

		Time			Treatment			Time x Treatment		
		ss	F	p	ss	F	p	ss	F	p
March 2007	Micrometazoans	63.53	0.69	0.633	75.90	3.19	0.149	120.15	1.31	0.298
df_{time} (5, 20)	Oligochaetes	1.42	1.00	0.443	0.284	1.00	0.374	1.42	1.00	0.443
$df_{treatment}$ (1, 4)	Platyhelminths	-	-	-	-	-	-	-	-	-
$df_{time\ x\ treatm}$ (5, 20)	Rotifers	17.18	0.30	0.903	98.74	10.27	**0.033**	80.98	1.44	0.252
May 2007	Micrometazoans	4,130.16	2.66	**0.040**	154.22	1.53	0.284	2,756.76	1.78	0.146
df_{time} (6, 24)	Oligochaetes	4.88	0.26	0.948	11.20	1.34	0.311	28.35	1.54	0.210
$df_{treatment}$ (1, 4)	Platyhelminths	135.05	3.26	**0.017**	0.50	0.01	0.925	156.57	3.79	**0.009**
$df_{time\ x\ treatm}$ (6, 24)	Rotifers	4,331.04	5.64	**0.001**	394.45	5.67	0.076	2,593.88	3.38	**0.015**
August 2007	Micrometazoans	110.65	0.41	0.832	0.55	0.01	0.951	159.54	0.60	0.701
df_{time} (5, 20)	Oligochaetes	10.46	0.87	0.519	0.02	0.01	0.922	22.12	1.84	0.151
$df_{treatment}$ (1, 4)	Platyhelminths	9.18	2.15	0.101	0.13	0.07	0.801	6.44	1.51	0.232
$df_{time\ x\ treatm}$ (5, 20)	Rotifers	70.48	1.36	0.280	6.60	0.64	0.469	33.01	0.64	0.673

In the last experiment (August 2007) total micrometazoan abundances were rather low, similar to the observations in March 2007, and not significantly affected by the BAC treatment neither regarding the total abundance of the micrometazoans, nor regarding distinct taxa. The only remarkable, though not significant, observation was an increase of rotifers in the BAC flow cells on the last day of this experiment (day nine).

Discussion

This work presents the first successful application of a novel type of flow cells, which facilitated the experimental manipulation of mature biofilm-dwelling consumer communities. The run-time of the experiments was sufficiently long to track population dynamics over several generations. Although the experiments were terminated when equilibrium abundances of the consumer communities were reached, the potential run-time of the flow cells could have been prolonged. The advantage of this method is the opportunity for live observations and thus the possibility to track short-term community dynamics. This allows disentangling specific processes, such as predator-prey dynamics, which could be overlooked on a broader spatial and temporal scale.

Regarding the supplemented resource, previous experiments (see Chapter 3) demonstrated that the same bacteria supplement as applied here (10^6 cells ml^{-1}) generates strong and sustaining effects on the quantity of early biofilm-dwelling ciliate communities, which showed similar final ciliate densities as the mature communities considered here. However, early biofilms hold much lower (if any) abundances of micrometazoans than mature biofilms do. The high metazoan abundance in the mature biofilms of the present study coincided with strong differences in the observed responses of ciliates to bacteria supplements compared to early communities: In all of the three experiments presented here, the final abundances were not different between the AMB and the BAC flow cells. As the resource levels were expected to suffice in

generating effects on the ciliate quantity (see above), it is likely, that elevated ciliate productivity was transferred to consumers. In fact, such assumption was confirmed by the predator-prey dynamics in March and in August 2007. In both experiments, initially stimulated ciliate abundances were followed by increased abundances of potential micrometazoan grazers. One key component among the micrometazoan community was rotifers; they were most probably responsible for a decrease of the initially shifted ciliate abundances in the supplemented flow cells back to the levels of the control biofilms in March 2007 and in August 2007. This finding (strong grazing pressure of rotifers on ciliates) was confirmed by live observations of especially brachionid rotifers grazing on colonial, non-loricated peritrichs (e.g. *Carchesium polypinum*, *Zoothamnium pectinatum*). Similar observations were made in previous experiments, where enhanced ciliate densities increased the abundance of rotifers which in return reduced the ciliates (see Chapter 3), too. This stresses the importance of interactions between rotifers and biofilm-dwelling ciliates like yet characterized for planktonic food webs only (Mohr and Adrian 2002). It would be worth to investigate, whether the rotifers profited from the enhanced ciliate productivity per se or if the previous uptake of the supplemented bacteria could have improved the nutrient value of the ciliates for the rotifers. Such couplings were reported for Daphnia when grazing on bacteria-fed ciliates (Martin-Creuzburg et al. 2006). However, such possibility is not supported by Breteler et al (2004) who found no trophic upgrading of copepods by ciliates that grazed on bacteria.

In May 2007, no effect of the resource supplement on the ciliate quantity was detected at all. Being the experiment with the highest *ab initio* density of micrometazoan grazers, it is likely that potentially enhanced ciliate productivity could have been transferred to a higher trophic level immediately, so that no quantitative effect on ciliates became obvious. This assumption is stressed by significant effects of the resource supplement on the micrometazoan density. In addition to the previously discussed experiments, this demonstrates that strong top-down pressure by grazers rather that bottom-up control by resources can

trigger the abundance of ciliates among biofilms. However, there were distinct adjustments in the taxonomic structure of the ciliate communities detected. In May 2007, for example, smaller peritrich ciliates were replaced by larger (grazing resistant) heterotrichs towards the end of experiment. It should be noted that an increased abundance of larger ciliates on biofilms could increase the resource density for higher metazoans such as snails. This should be a subject of further investigations. However, the strongest responses of ciliate taxa to the resource supplements were detected among the smaller peritrichs. Thereby, it is remarkable that the strongest short-term response of the ciliate communities to the resource supplement was detected in March 2007 incorporating low ambient water temperatures ($<10^{o}C$). This suggests that at least temperature was no factor in determining the magnitude of community responses to increased bacterial densities. However, this observation showed, that when the ambient resource load is exceeded, ciliate communities can rapidly respond to enhanced densities of planktonic resources with enhanced productivity.

Conclusion

The application of the new flow cell method presented here revealed important insights in the short-term dynamics of biofilm-dwelling consumer communities in response to short-term resource increases. The obtained results add an important aspect to the current knowledge on the control of biofilm-dwelling ciliates communities, i.e. the predominant top-down control of mature ciliate communities on biofilms by micrometazoans. However, bottom-up effects may persist and result in altered community compositions at though constant (top-down controlled) total ciliate abundances. This work demonstrates both the complexity of food webs on biofilms (incorporating several trophic levels), and that resource effects on biofilm-dwelling consumer communities might be hidden due to a rapid transfer of enhanced ciliate productivity to higher trophic levels. It further demonstrated the necessity for a differentiated view of bottom-

up vs. top-down controlling factors with respects to "producers" and "consumers".

Concluding remarks and perspective

Although biofilm-dwelling ciliate communities are an important component of aquatic food webs, little is known on how external factors can control their community structure on both temporal and spatial scales. The present study contributes to the understanding of biofilm-dwelling ciliate community responses towards environmental changes.

The first part of the study concentrated on investigating the influence of temperature on the development of ciliate communities in the context of global warming. This was done by manipulating the water temperature of naturally grown, biofilm-dwelling ciliate communities. The experiment was designed to allow the seasonally dependent responses of the ciliate community to be assessed. These responses were strongest for the more extreme seasons (winter and summer).

It was shown that temperature increase during winter can significantly accelerate the colonization of biofilms by ciliates. This can further result in the development of significantly altered ciliate communities due to a symmetric growth enhancement of the dominant ciliate taxa. Such a strong response towards warming can be due to a high resource density during winter, enabling enhanced ciliate growth. This finding was partially supported by the experiments with mature biofilm communities; a general enhancement and a differentiation of the ciliate communities (corresponding to a non-symmetric enhancement of particular ciliate taxa) due to warming was also observed here. This demonstrates that increasing temperature can sustainingly influence the community composition. However, this finding that ciliate productivity can profit from increasing temperature contrasted the expectations based on the metabolic theory: At constant resource supply, warming should decrease the carrying capacity to balance increased metabolic costs. Such results were obtained by temperature increases during summer. While the colonization rate of biofilms by ciliates was not affected by warming, the carrying capacity of the communities significantly decreased at elevated temperatures. This decrease, however, did not result from the exclusion of particular ciliates and there was no

shift in the ciliate community structure, showing that the ciliates in this experiment were still inside their thermal niche even at (for the River Rhine) very high temperatures of >30oC. It was further shown that negative impacts of warming on ciliate communities can be buffered by supplemental resources. This stresses that energetic factors were the main reason for negative effects of warming on ciliate communities rather than thermal limitations of the ciliates during summer. Further investigations should concentrate on possible microevolutionary responses of the ciliates beyond the microscopically detectable taxonomic level.

As the "temperature experiments" demonstrated that the structure of biofilm-dwelling ciliate communities is strongly influenced by seasonal variability (particularly the resource density), the second part of this study investigated the effects of resource quantity on the development and on the structure of ciliate communities. Resources from two different origins were used, namely benthic and planktonic bacteria. To my knowledge, this is the first study conducted to investigate the effects of controlled resource enhancements on natural ciliate communities.

The stimulation of benthic bacterial growth with labile organic carbon generally resulted in the accumulation of filamentous bacteria (inedible for ciliates) towards the end of the experiments rather than in a significant response of the ciliates. However, a few ciliates (which are typical pioneers on biofilms) profited from the enhanced benthic bacterial growth within the early phase of biofilm development. An indirect pathway of increased benthic bacterial biomass (stimulation of heterotrophic flagellates and adjacent grazing by ciliates) only occurred at higher temperatures. Stronger responses were generally obtained by raising the density of planktonic bacteria. As expected, planktivorous ciliates significantly profited from this resource supplement, whereas there was no general response detected among benthivorous ciliates. The significant exception was an indirect stimulation of stichotrichous ciliates; they responded to increased abundance of heterotrophic flagellates which was strongest during the colder seasons. These findings demonstrate the necessity for a seasonally

differential view of "resources" for biofilm-dwelling ciliates, with particular regards to the origin (planktonic versus benthic) of the resources. Thereby it was shown that heterotrophic flagellates (being important vectors for energy in the plankton) can be important mediators for energy in benthic habitats as well. Further work should concentrate on competitive patterns between heterotrophic flagellates and ciliates with regards to seasonal dependencies of the trophic transfer.

Another important factor for the magnitude of community responses are micrometazoan grazers: Increased ciliate productivity can be transferred to grazers immediately so that no sustainable effect on the ciliate communities would become obvious. However, the results of the experiments indicated that smaller ciliates may be replaced by larger ciliates after grazing. This finding could have further implications for specific trophic feedbacks between particular ciliates and grazers. Whereas high densities of smaller ciliates could enhance micrometazoan grazers, larger ciliates could increase the nutrient value of the biofilms for larger metazoan grazers (e.g. insects, snails). Further work should concentrate on this topic.

Taken together, the different aspects of the present work demonstrate the importance of temperature, resource density and grazer density in controlling biofilm-dwelling ciliate dynamics. Although each factor can significantly affect the communities, it is the interaction between these factors that controls the communities in the end. The strength of bottom-up (e.g. resource density) vs. top-down (e.g. grazers) factors thus depends on the particular environmental conditions. However, the high capacity of biofilm-dwelling ciliate communities to respond to environmental changes (stimulation of the ciliates by temperature and/or resources and trophic upgrading of the biofilms for grazers) generally observed in this study emphasises the ecological significance of ciliates in transferring otherwise unavailable energy to higher trophic levels in benthic habitats.

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Erweiterte Zusammenfassung

Mikrobielle Lebensgemeinschaften spielen im Stoffumsatzgeschehen aquatischer Ökosysteme eine wichtige Rolle. Insbesondere in Flach- und Fliessgewässern findet der größte Teil mikrobieller Aktivität auf oder in Assoziation mit Oberflächen statt. Auf diesen so genannten Biofilmen dominieren unter den Konsumenten häufig Ciliaten hinsichtlich der Biomasse (Gong et al. 2005), die neben makrobenthischen Invertebraten (z.B. Muscheln; vgl. Welker & Walz 1998) wichtige Importeure organischen Materials aus dem Plankton in das Benthos sein können (vgl. Weitere *et al.* 2003). Ciliatengemeinschaften setzen sich aus einer Vielzahl von Taxa zusammen, die neben ihrer Größe (ca. 10-2000µm) ebenso stark in ihrer ökologischen Funktion variieren. Die Funktionalität von Ciliatengemeinschaften auf Biofilmen als Gesamtheit hängt daher neben der Organismendichte (Abundanz, Biovolumen) auch von der Gemeinschaftsstruktur ab, die sowohl in zeitlicher als auch in räumlicher Hinsicht durch zahlreiche Faktoren beeinflusst wird.

Während die Steuerung planktischer Ciliatengemeinschaften im Laufe der letzten Jahrzehnte gut untersucht wurde, weiß man bisher wenig darüber, welche Faktoren an der Steuerung Biofilm bewohnender Ciliatengemeinschaften beteiligt sind. Ein Grund ist die schlechte experimentelle Zugänglichkeit von Biofilmen. Verschiedene Techniken zur experimentellen Analyse von Wirkungszusammenhängen in planktischen Nahrungsnetzen (z.B. Größenfraktionierungen, Verdünnungsexperimente) lassen sich schlecht auf Biofilme übertragen. Mit der Ökologischen Rheinstation, Köln-Bayenthal, besitzt die Universität zu Köln eine seltene Möglichkeit, ökologische Experimente mit naturnah gewachsenen Biofilmgemeinschaften durchzuführen. Die Biofilme werden dabei in flusswassergespeisten Bypasssystemen kultiviert. Das Flusswasser ist sowohl Träger der Organismen (vgl. Scherwass & Arndt 2005) die sich auf den Biofilmen ansiedeln, als auch das für die Erhaltung der Biofilmgemeinschaften essenzielle Ressourcenreservoir In diesen Bypasssystemen lassen sich

einzelne Umweltparameter gezielt manipulieren und so deren Einflüsse auf Gemeinschaften untersuchen.

Der Bedeutung von Temperaturerhöhungen für ökologische Gemeinschaften gilt heute enorm gesteigertes wissenschaftliches und öffentliches Interesse. Aus anthropogen verursachten Temperaturanstiegen (vgl. IPCC 2007) könnten sich bislang unabschätzbare Konsequenzen für ökologische Gemeinschaften ergeben, die sich experimentell nur schwierig untersuchen lassen. Da Temperatureffekte auf Gemeinschaften erst im Laufe vieler aufeinander folgender Generationen auftreten können, sind mikrobielle Lebensgemeinschaften ein wichtiges Modellsystem: Aufgrund ihrer enorm hohen Wachstumsraten mit Generationszeiten (Stunden bis Tage) lassen sich komplexe Wirkungszusammenhänge zwischen Umweltfaktoren und Populationsdynamiken innerhalb relativ kurzer Zeit testen.

Mittels rheinwasserdurchströmten Miniaturfliesszellen wurde der Einfluss lokaler Temperaturerhöhungen auf die frühe Entwicklung von Ciliatengemeinschaften auf Biofilmen (ausgehend von sterilen Oberflächen) bis zum Erreichen eines frühen Besiedlungsplateaus (Kapazität) untersucht, bei dem solitäre Ciliaten in der Abundanz überwiegen. Dieses grenzt sich damit klar von einem späten Besiedlungsplateau ab, bei dem koloniale Ciliaten dominieren. Die Fliesszellen wurden ausgehend von einer Grundtemperatur T_0 (auf Basis der langjährigen Monatsmitteltemperaturen) bei verschiedenen Temperaturenerhöhungen gehältert. Es zeigte sich, dass Temperatur-erhöhungen im Winter zu einer signifikant beschleunigten Biofilmbesiedlung durch Ciliaten führen können, die nach zehntägiger Entwicklung ebenso zu einer Ausprägung signifikant verschiedener Ciliatengemeinschaften in Folge symmetrisch erhöhter Abundanzen der dominierenden Ciliatentaxa führten. Anschließend wurde getestet, ob diese Beobachtung auf späte Ciliatengemeinschaften übertragen werden kann. Dazu wurden Ciliatengemeinschaften für acht Wochen auf Objektträgern bei zwei Temperaturen (T_0; $T_0+3^{\circ}C$) in Fliessrinnen kultiviert. Ähnlich dem Kurzzeitversuch war das Gesamtbiovolumen der Ciliaten bei Versuchsende

signifikant erhöht. Ebenso bildeten sich unterschiedliche Ciliaten-gemeinschaften in Folge von Erwärmung aus. Anders als im Fliesszellenexperiment mit jungen Biofilmen war dies jedoch durch eine Verschiebung in der taxonomischen Struktur der Ciliatengemeinschaften verursacht. Die beobachteten positiven Effekte auf die Kapazität von späten Ciliatengemeinschaften im Winter standen im Gegensatz zu den Erwartungen basierend auf der metabolischen Theorie. Danach sollte sich die Kapazität von Gemeinschaften bei unveränderter Ressourcendichte durch Erwärmung vermindern (vgl. Brown *et al.* 2004, Savage *et al.* 2004).

Im Sommer führte die lokale Erwärmung zu gegensätzlichen Effekten. Während sich die Besiedlungsgeschwindigkeit von Ciliaten nicht erhöhte, wurde die Kapazität der Biofilme für Ciliaten signifikant reduziert. Gleichzeitig gab es keine temperaturbedingte Ausprägung unterschiedlicher Ciliaten-gemeinschaften. Anschließend wurde getestet, ob der negative Erwärmungseffekt auf die Ciliatengemeinschaften durch erhöhte Ressourcen-verfügbarkeit abgemildert werden kann. Die dazu durchgeführten Experimente mit Kreuzmanipulation von Temperatur ($+3^{\circ}C$) und erhöhter Ressourcenverfügbarkeit zeigten, dass die frühe Biofilmbesiedlung durch Ciliaten durch Erhöhung beider Komponenten im Sommer signifikant beschleunigt wird. Darüber hinaus kann der negative Effekt von Erwärmung (bei konstanter Ressourcenverfügbarkeit) auf die Kapazität von Ciliatengemeinschaften durch zusätzliche Ressourcen kompensiert werden. Daher sind energetische Ursachen (keine Kompensation temperaturbedingt erhöhter metabolischer Aktivität durch gesteigerte Nahrungsaufnahme) wahrscheinlich die Hauptursache für negative Gemeinschaftseffekte durch Erwärmung im Sommer gewesen.

Zusammenfassend zeigten die Versuche, dass Einfluss von Temperaturerhöhungen auf Ciliatengemeinschaften sowohl hinsichtlich der quantitativen als auch der qualitativen Gemeinschaftstruktur deutlich von den Umweltrahmenbedingungen (insbesondere der Ressourcen-Verfügbarkeit).

Tabelle 1. Einfluss erhöhter Temperaturen im Winter und Sommer (ohne, mit Ressoucenerhöhung) auf die Besiedlungsgeschwindigkeit und die Kapazität von Ciliatengemeinschaften. (⇑) Erhöhung, (⇓)Verminderung, (⇒) keine Änderung. Gestrichelte Pfeile symbolisieren nichtsignifikante Gemeinschaftseffekte.

|	Winter	Sommer	Sommer (+Ressourcen)
Besiedlungsgeschwindigkeit	⇑	⬈	⇑
Kapazität	⇑	⇓	⇢

Die vorangestellten Versuche hatten gezeigt, dass die Struktur von Ciliatengemeinschaften neben der Temperatur ebenfalls stark von der Ressourcendichte beeinflusst wird. Der Begriff „Ressource" ist dabei für biofilmbewohnende Konsumenten häufig nicht klar definiert. Ähnlich der taxonomischen Diversität weisen Ciliaten eine ebenso hohe funktionelle Vielfältigkeit auf. Viele Ciliaten sind planktivor und nehmen Partikel hauptsächlich aus der Wassersäule auf. Andere Ciliaten sammeln Partikel von der Oberfläche ab oder „jagen" ihre Beute. Neben der Ressourcendichte kann daher auch die Art der Ressource für die Steuerung von Ciliatengemeinschaft von Bedeutung sein. In den nachfolgenden Experimenten wurde deshalb die Bedeutung von Ressourcen unterschiedlichen Ursprungs für die frühe Entwicklung von Ciliatengemeinschaften getestet. Die benthische Ressourcenverfügbarkeit wurde durch kontinuierliche Zufütterung von Hefeextrakt (Stimulation benthischen bakteriellen Wachstums) zu Biofilmen in Miniaturfliesszellen erhöht. In weiterer Experimenten wurde die planktische Ressourcenverfügbarkeit wurde durch Zugabe von Bakterien (*Pseudomonas putida*) zu den Fliesszellen manipuliert.

Beide Ressourcenmanipulationen beeinflussten die Gesamtabundanz als auch die Struktur der Ciliatengemeinschaften in Abhängigkeit des Entwicklungs-stadiums der Biofilme. Dabei waren die Effekte erhöhten benthischen

bakteriellen Wachstums eher schwach: Nur in einem Experiment (Sommer 2007) wurde die Biofilmbesiedlung durch Ciliaten erhöht, die Folge einer Stimulation gleitender bakteriovorer Flagellaten war, welche nanophage Ciliaten konsumieren konnten. Meistens führte diese Art der Ressourcenmanipulation zur Bildung fraßresistenter Bakterienfilamente.

Die Effekte erhöhter planktischer Bakteriendichte waren ebenfalls zweischichtig: Neben der erwarteten direkten Ausnutzung durch planktivore Ciliaten (z.B. Choreotrichia, Peritrichia, Scuticociliatia) bei höheren Temperaturen kam es vor allem bei niedrigen Temperaturen zu einer signifikanten Stimulation planktivorer heterotropher Flagellaten. Diese konnten die erhöhte Bakteriendichte in Folge höherer Wachstumsraten im Vergleich zu den Ciliaten besser ausnutzen. Die Flagellaten wurden anschließend von Ciliaten (insbesondere Stichotrichia) abgeweidet, was zu einer indirekten Stimulation der Ciliatengemeinschaft führte. Neben einer signifikant beschleunigten Biofilmbesiedlung war die Maximalabundanz der Ciliaten in jedem Experiment gegen Versuchende signifikant erhöht. Die Ausnahme bildete das Frühjahrsexperiment: Hier wurde die Maximalabundanz bereits nach fünf Tagen erreicht, bevor die Abundanz erneut bis auf das Niveau der Kontrollen abfiel. Diese Beobachtung deckte sich mit einer deutlichen erhöhten Abundanz von Rotatorien, welche die erhöhte Ciliatenbiomasse vermutlich konsumierten.

Die Effektstärke beider Ressourcenerhöhungen hing stark von dem Versuchszeitpunkt ab und verdeutlicht die Möglichkeit saisonaler Unterschiede in der Ressourcennutzung durch Ciliatengemeinschaften in Abhängigkeit der natürlichen Rahmenbedingungen respektive der Temperatur (vgl. Abb. 2), der natürlichen Ressourcendichte sowie der Konsumentenaktivität. Darüber hinaus können Stimulationen bestimmter taxonomischer Gruppen jahreszeitlich variieren, sodass mögliche Einflüsse auf die Struktur von Gemeinschaften nicht grundsätzlich aus der Ressourcendichte vorausgesagt werden können.
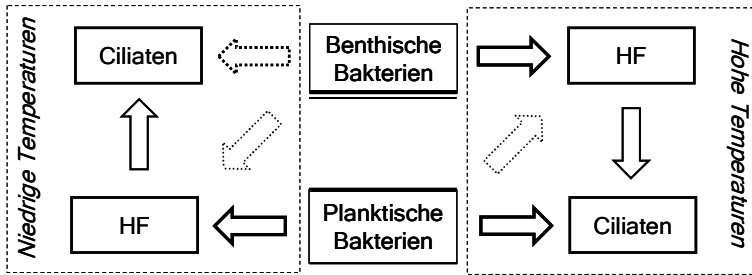
Abb. 1. Schema der Hauptnutzungswege erhöhter benthischer und planktischer Ressourcen durch biofilmbewohnende Ciliatengemeinschaften bei (links) niedrigen und (rechts) hohen Temperaturen. Unterschiedliche Pfeildicken verdeutlichen die beobachteten Effektstärken.

Nachdem gezeigt wurde, dass insbesondere eine erhöhte planktische Bakteriendichte effektiv von Ciliatengemeinschaften genutzt werden kann, wurde im letzten Versuchsteil untersucht, inwiefern eine Erhöhung der Bakteriendichte durch später Ciliatengemeinschaften ausgeschöpft werden und möglicherweise zu Änderungen in der Gemeinschaftsstruktur führen kann. Dazu wurde ein neues Fliesszellensystem konstruiert, welches die Manipulation sowie die mikroskopische Direktbeobachtung zuvor im Fluss exponierter Ciliatengemeinschaften ermöglicht. Zu drei Versuchszeitpunkten (März, Mai, August 2007) wurden Ciliatengemeinschaften für zwei Monate in Fliessrinnen vorkultiviert und anschließend in Fliesszellen mit und ohne Zufütterung planktischer Bakterien (*P. putida*) überführt.

Alle drei Versuche ergaben ähnliche Resultate. Neben einer Stimulation der Ciliatengemeinschaften kam es zu einer Erhöhung von Konsumenten, welche die erhöhte Ciliatendichte konsumierten, sodass bei Versuchende kein Unterschied zwischen zugefütterten und nicht zugefütterten Gemeinschaften mehr festzustellen war. Im Mai 2007 wurde keine signifikante Änderung der Ciliatendichte durch die Ressourcenerhöhung festgestellt. Dies könnte auf einer in diesem Experiment beobachteten hohen Mikrometazoendichte beruhen, die eine potentiell erhöhte Ciliatenproduktion direkt konsumieren konnten. Diese Ergebnisse zeigen, dass die Struktur später (reifer)

Ciliatengemeinschaften in viel stärkerem Maße top-down (durch Konsumenten) kontrolliert sind als bottom-up durch die Ressourcenverfügbarkeit.

Zusammengefasst zeigen die hier vorgestellten Arbeiten, dass beide untersuchten Faktoren - Temperatur und Ressourcenverfügbarkeit - die Entwicklung und die Struktur biofilmbewohnender Ciliatengemeinschaften maßgeblich beeinflussen; die Reaktionsstärke der Gemeinschaften hing dabei von weiteren Umweltparametern ab, insbesondere von der natürlichen Ressourcendichte (bei experimenteller Temperaturerhöhung) sowie von der Wassertemperatur (bei experimenteller Ressourcenerhöhung). Das zeigt, dass Temperatur und Ressourcenverfügbarkeit bei der Steuerung von Ciliatengemeinschaften stark interagieren. Voraussagen über Gemeinschaftseffekte infolge von Umweltveränderungen müssen jedoch stets jahreszeitliche Variabilität in den Umweltrahmenbedingungen (Temperatur, Ressourcendichte, Konsumentenaktivität) mit einbeziehen.

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Kurzzusammenfassung

Bei den hier vorgestellten Arbeiten wurde der Einfluss von Temperatur und Ressourcenerhöhung auf die Entwicklung von biofilmbewohnenden Ciliatengemeinschaften untersucht. Dazu wurden experimentelle Labor- und Freilandansätze miteinander kombiniert.

Der erste Teil dieser Arbeit befasste sich mit dem Einfluss experimenteller Erwärmung auf Ciliatengemeinschaften. Es zeigte sich, dass eine Temperaturerhöhung im Winter die Biofilmbesiedlung durch Ciliaten sowohl signifikant beschleunigen als auch die maximale Ciliatendichte erhöhen kann, insofern ausreichend Ressourcen vorhanden sind. Dies führt ebenfalls zu einer Ausprägung signifikant verschiedener Ciliatengemeinschaften in Folge von Erwärmung. Im Gegensatz dazu führt Temperaturerhöhung bei niedrigen Ressourcendichten im Sommer zu einer reduzierten Kapazität von Biofilmen für Ciliaten. Die Bedeutung der Ressourcenverfügbarkeit konnte in einem Experiment mit Kreuzmanipulation (Temperatur- und Ressourcenerhöhung) bestätigt werden, in welchem die negativen Einflüsse von Erwärmung auf die Kapazität durch zusätzliche Ressourcen kompensiert werden konnten.

Der zweite Teil dieser Arbeit befasste sich mit dem Einfluss von erhöhter Ressourcenverfügbarkeit (planktische und benthische Bakterien) auf Ciliatengemeinschaften. Dabei fielen die Effekte erhöhter benthischer Bakteriendichte auf die Ciliatengemeinschaften eher gering aus, wo hingegen die Gemeinschaften von einer erhöhten planktischen Bakteriendichte generell deutlich profitieren konnten. Insbesondere bei hohen Temperaturen war die Stimulation der Gemeinschaften Folge einer direkten Erhöhung planktivorer Ciliaten, während bei niedrigen Temperaturen eine indirekte Förderung der Ciliaten auftrat. Diese war an ein erhöhtes Wachstum suspensionsfressender heterotropher Flagellaten gekoppelt, die anschließend von Ciliaten abgeweidet wurden. Die Effektstärken hingen dabei stark von den Umweltrahmen-bedingungen sowie von dem Vorhandensein von Konsumenten der Ciliaten ab. Zu Letzterem konnte in weiteren Versuchen mit späten, vorkultivierten Biofilmen gezeigt werden, dass erhöhte Ciliatenproduktion in Folge von

Ressourcenerhöhungen direkt auf Konsumenten übertragen werden können, sodass kein quantitativer Effekt auf die Ciliatengemeinschaft verzeichnet werden kann.

Zusammengefasst zeigen die hier vorgestellten Arbeiten, dass Temperatur und Ressourcenverfügbarkeit die Entwicklung von biofilmbewohnenden Ciliatengemeinschaften separat betrachten signifikant beeinflussen können. Die Stärke von Gemeinschaftseffekten hing dabei jedoch immer von weiteren Umweltparametern ab, insbesondere von der Ressourcenverfügbarkeit (bei experimenteller Temperaturerhöhung) und von der Temperatur (bei experimenteller Ressourcenerhöhung. Das zeigt, dass Temperatur und Ressourcenverfügbarkeit bei der Steuerung biofilmbewohnender Ciliatengemeinschaften stark interagieren. Allerdings können die Effekte von Umweltveränderungen auf Ciliatengemeinschaften von Konsumenten der Ciliaten überlagert werden. Voraussagen über Anpassungen von Gemeinschaften infolge von Umweltveränderungen müssen daher stets jahreszeitliche Variabilität (Temperatur, Ressourcenverfügbarkeit, Konsumentenaktivität) mit einbeziehen.

Abstract

Laboratory and field-related experimental approaches were combined to investigate the impacts of temperature and resource enhancements on the development of biofilm-dwelling ciliate communities.

The first part of this study concentrated on ciliate community responses towards experimental warming. It was shown that temperature increase during winter can significantly accelerate the early colonization of biofilms by ciliates and enhance the organism density when the resource supply is sufficient. This can also result in the formation of significantly altered ciliate communities in consequence to temperature increases. In contrast, temperature increase during summer reduces the carrying capacity of biofilms for ciliates when the resource density is low. This finding was confirmed by the results of an experiment with cross-manipulations (temperature- and resource enhancements), in which the negative effect of warming was buffered by supplemental resources
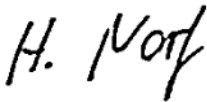
The second part of this work concentrated on the responses of biofilm-dwelling ciliate communities towards resource enhancements from two different origins, namely benthic and planktonic bacteria. It was shown that ciliate community responses towards benthic bacteria enrichments are often limited, whereas the ciliates can generally profit from planktonic bacteria enhancements. Such stimulation could either occur directly by the enhancement of suspension-feeding ciliates at especially high temperatures, whereas indirect ciliate community responses were detected, especially at low temperatures. Here, their enhancement was coupled to a previous enhancement of suspension-feeding heterotrophic flagellates, which in return were grazed upon by ciliates. The magnitude of responses strongly depended on the seasonal conditions with regards to both the environmental setting as well as to the presence or absence of ciliate consumers (micrometazoa). The latter finding was also confirmed for pre-grown, mature ciliate communities.

Taken together, the different aspects of this study demonstrated that when considered separately, both factors (i.e. temperature and resource density) can

significantly affect the development and the structure of biofilm-dwelling ciliate communities. However, the magnitude of community responses towards manipulations of either factor was tightly coupled to the environmental conditions with particular regards to the ambient resource load (in the experiments with temperature enhancements) and to the ambient water temperature (in the experiments with resource enhancements). This demonstrates that temperature and resource availability interactively control the development and the structure of biofilm-dwelling ciliate field communities. Admittedly, ciliate community responses to environmental changes can be hidden due to grazer activities. Although, the assumption of community responses towards environmental changes always has to consider the environmental background (temperature, resource availability, grazer activity) besides of shifts in particular variables.

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Abbildungen und Tabellen - die anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzellfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von den auf der folgenden Seite angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Hartmut Arndt betreut worden.

Köln, im April 2008

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[4]Norf H, Arndt H, Weitere M - Impacts of winter vs. summer warming scenarios empirically tested using open ciliate biofilms. Eingereicht bei *FEMS Microbiology Ecology*.

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[1] Inhaltlich Kapitel 3 der Dissertation

[2] Inhaltlich Kapitel 1 der Dissertation

[3] Inhaltlich Kapitel 4 der Dissertation

[4] Inhaltlich Kapitel 2 der Dissertation