Rapid Daphnia-mediated changes in microbial community structure: an experimental study

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Abstract

Shifts in morphological and taxonomical composition of bacterioplankton communities in response to protist and metazoan grazing were studied in bottle experiments, exposing bacterioplankton from a eutrophic clear-water pond, dominated by a large population of Daphnia magna, to a Daphnia gradient, ranging from 0 to 60 individuals per liter. Prior to the first experiment, the bacterioplankton community was shaped by protist grazing, while for the second experiment, bacterioplankton was pre-adapted to Daphnia grazing. In both experiments, rapid shifts in biomass and structure of the bacterioplankton community upon exposure to Daphnia grazing were observed. High Daphnia densities suppressed protozoa, resulting in a dominance of free-living bacteria. Under low Daphnia densities, heterotrophic nanoflagellates (HNF) developed as the dominant grazers and complex morphotypes (filaments, aggregates) were abundant in the bacterial community. Denaturing gradient gel electrophoresis analysis showed that taxonomical changes accompanied the morphological differences between bacterial communities shaped by HNF or Daphnia grazing. However, comparing ciliate- and Daphnia-dominated bacterial communities, we observed a discrepancy between morphological and taxonomical shifts, indicating that other traits than mere morphological ones determine vulnerability of bacterioplankton to specific grazers. Our results illustrate the rapid, pronounced and reversible impact of grazing on the morphology and taxon composition of bacterioplankton. Our results also stress that Daphnia may, already at moderate densities, have a pronounced impact on the lake bacterioplankton, both through direct grazing on the bacteria and through grazing on protozoan bacterivores. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Daphnia grazing; Bacterioplankton; Morphotype; DGGE

1. Introduction

Bacteria are a functionally and quantitatively significant component of aquatic food webs. They are the primary decomposers of allochtonous and autochtonous organic material and mineralizers of organic matter into inorganic compounds that can be used by phytoplankton for primary production [1]. As prey item of many organisms, they may also be an important trophic link in aquatic food webs, because they transform dissolved organic matter that is excreted by photosynthetically active phytoplankton into particles that can be transferred to higher trophic levels. Factors that regulate the abundance, distribution, growth rate, and respiration of microorganisms thus influence some of the key functions of aquatic ecosystems. Biomass and growth rate of planktonic bacteria are regulated by the availability of inorganic and organic nutrients [2], protozoan and metazoan grazing [3,4], and viral lysis [5,6]. Predation is generally considered as the main factor controlling the biomass standing stock of bacterial communities [2,7]. Protists, especially heterotrophic nanoflagellates (HNF), have been reported as the principal consumers of bacteria in most aquatic ecosystems [3]. Many of these HNF lack elaborate feeding structures and they capture and process single prey particles, which makes morphological and chemical properties of bacteria...
important for their selective prey uptake [8]. With respect to size-selective bacterivory, there is now solid evidence that HNF grazing results in the preferential removal of relatively large, actively growing and dividing bacteria [9,10], while ‘very small’, often inactive cells can persist in the community [11,12]. Moreover, HNF grazing may, especially in more productive freshwater and coastal environments [13,14], also result in the development of complex grazing-resistant bacterial (GRB) morphotypes (filaments and aggregates) that are too large to become ingested [15–18]. Consequently, HNF grazing pressure may shift the bacterial assemblage bi-directionally towards grazing-resistant cells in the smaller and larger size classes [16,19]. These morphological shifts in the bacterial community can reflect the rise of specific genotypes that adopt distinct strategies in reaction to HNF grazing [17,18,20,21] or may reflect high potential for phenotypic plasticity [22].

Although HNF are often considered to be the most important bacterivores, several studies have reported that ciliates can also be important consumers of planktonic bacteria [23,24]. In addition, large filter-feeding cladocerans of the genus *Daphnia*, that can achieve high population densities in eutrophic freshwater systems, may become the major bacterial consumers [25]. Studies on *Daphnia* grazing on bacteria have focused mainly on quantitative aspects [26] and much less is known about qualitative effects on bacterial community structure. There is some evidence that bacterial morphological defences against protist grazing contrast those that are effective against grazing by *Daphnia* [13,26]. Based on these literature data, we anticipated that the impact of *Daphnia* on the bacterioplankton would be very different depending on whether the bacterial community was exposed (and adapted) to HNF grazing prior to the appearance of *Daphnia*.

Our experiments aimed to study the shifts in bacterial community structure and composition when main grazing pressure changes from HNF to *Daphnia*. This was studied with different *Daphnia* densities and bacterial communities, which have either developed during HNF grazing or during *Daphnia* grazing. As we combined morphological characteristics of the bacterial community with molecular analysis of taxonomical composition, our analysis also reveals the extent to which morphological changes in the grazer-exposed bacterial community involve changes in taxonomic composition.

2. Materials and methods

The experiments were run during May 2000 with water from a shallow, eutrophic pond in Northern Germany (Lebrader Teich, mean depth = 0.5 m, Table 1), which remains in a clear-water state during most of the season due to a relatively low fish stock and high densities of *Daphnia magna* (Jürgens, unpubl.).

2.1. Pre-experimental incubation

Three days before the start of the experiment, pond water was sampled and filtered through a 200-μm net to remove the mesozooplankton, which consisted mainly of daphnids. The water was transferred to the laboratory and incubated in a 60-l tank at 20°C and dim light conditions. Every 12 h, a subsample was taken from the water, stained with 4,6-diamidino-2-phenylindole (DAPI), and screened for the presence of microorganisms via epifluorescence microscopy. Right after transportation to the laboratory, very low densities of HNF and ciliates were present in this water, and free rods and cocci dominated the bacterial community. Three days after the elimination of the mesozooplankton, a characteristic microbial succession had occurred [26], in which HNF densities increased, followed by the increase of complex bacterial morphotypes like filamentous bacteria and aggregates. The bacterial community that developed was assumed to be shaped mainly by HNF grazing.

2.2. Experimental set-up

After the 3 days of pre-incubation, experiment 1 was started. Sixteen 1-l glass bottles were filled with 750 ml of the preconditioned, mesozooplankton-free lake water. From every bottle, samples for the enumeration of bacteria, HNF, ciliates and denaturing gradient gel electrophoresis (DGGE) were taken (a total volume of 250 ml was removed). Adult *D. magna*, sampled from the same lake the day prior to the experiment, were added to each bottle. There were eight treatments that made up a *Daphnia* gradient (0, 6, 10, 20, 30, 40, 50, 60 *Daphnia* l⁻¹). All treatments were done in duplicate. The bottles were incubated at slow rotation on a rolling table (ca. 0.5 rpm) in an acclimatized room (20°C) for approximately 3 days. At regular intervals, samples were taken for the quantification of bacteria, HNF and ciliates. After 67 h, the *Daphnia* of each bottle were isolated, fixed with ethanol (50%), and stored until further processing. Experiment 2 was analogous to experiment 1 but the pond water was not incubated prior to the experiment, thus exposing for 59 h a *Daphnia*-adapted bacterial community, directly taken from the pond, to the *Daphnia* gradient.

2.3. Microscopical processing of the samples

For enumeration of microorganisms, 10-ml samples were fixed with formalin (2% final concentration) and stored at 4°C. Of each replicate, 1 ml subsamples were stained with DAPI (final concentration 100 μg ml⁻¹ [27]), filtered on a black 0.2-μm polycarbonate filter, and analyzed with fluorescence microscopy (at 1250× magnification). Mean biovolumes of free rods and cocci were determined from cell dimensions using an automated image analysis system (SIS GmbH, Münster, Germany) with
Table 1

| Nutrient concentrations (µg l⁻¹) in Lebrader Teich on the moment of the experiments |
|------------------------------|------------------|
| Total P                      | 235              |
| PO₄-P                        | 165              |
| NH₄-N                        | 90               |
| NO₃-N                        | 9                |
| NO₂-N                        | 94               |
| Total diss. N                | 1464             |

Table 1: Nutrient concentrations (µg l⁻¹) in Lebrader Teich on the moment of the experiments.

an image processing and analyzing procedure similar to the one described by Massana et al. [28]. From every filter, randomly chosen sections were inspected and HNF and different prominent bacterial morphotypes were quantified separately. In addition to rods and cocci and dividing bacteria, specific morphotypes that were assumed to be less vulnerable to HNF (GRB) were distinguished: curve-shaped bacteria (‘bea-like bacteria’), filamentous forms (chains > 5 µm) and bacterial aggregates (containing ≥ 10 cells) [17]. Filament length and width were determined and filament biovolume was calculated, assuming filaments to be cylinders with hemispherical ends. The number of cells per aggregate was counted, or for aggregates with more than 100 cells, estimated, and the largest number of cells per aggregate was counted, or for aggregates to be cylinders with hemispherical ends. The termined and filament biovolumewascalculated,assuming

...ing patterns of the different samples was calculated from the increase or decrease in abundance during the incubations, assuming an exponential model.

2.4. DGGE analysis

For the analysis of bacterial community structures, we used DGGE [30]. 200-ml subsamples of every treatment from the start and end of the incubation were filtered on a polycarbonate membrane filter (pore size 0.22 µm; filter diameter 47 mm; Millipore). The filter was cut in four pieces with a sterile scalpel and each quarter was stored at −80°C until further processing. DNA was extracted directly using the bead-beating method concomitant with phenol extraction and ethanol precipitation [31]. Following extraction, the DNA was purified on a Wizard column (Promega, Madison, WI, USA) according to the manufacturer’s recommendations. A 16S rDNA fragment was amplified with primers, specific to the domain Bacteria (357F-GC-clamp (5’-CGCCCGCGCGCGCGCGCGCGCGCGCGCGCGC-GGGCCCGCGCCCGCGCGCGCGCGCGCGCGCGCGCGCCGGAGGCA-GCAG-3’) and 518R (5’-ATTACCGCGCTGCTGG-3’)). PCR amplification procedures were performed with a Genius temperature cycler. Each mixture containing 5 µl of template DNA, each primer at a concentration of 0.5 µM, each deoxynucleosidetriphosphate at a concentration of 200 µM, 1.5 mM MgCl₂, 20 ng of bovine serum albumin, 5 µl of 10× PCR buffer (100 mM Tris–HCl (pH 9), 500 mM KCl), and 2.5 U of Taq DNA polymerase (Ampli-Taq Perkin Elmer) was adjusted to a final volume of 50 µl with sterile water (Sigma). Following an incubation of 5 min at 94°C, a touchdown PCR was performed using 20 cycles consisting of denaturation at 94°C for 1 min, annealing at 65°C (the temperature was decreased by 0.5°C every cycle until the touchdown temperature of 56°C was reached) for 1 min, and primer extension at 72°C for 1 min. Five additional cycles were carried out at an annealing temperature of 55°C. The tubes were then incubated for 10 min at 72°C. PCR samples were loaded onto 42% polyacrylamide gels, 1 mm thick, in 1× TAE (20 mM Tris–acetate (pH 7.4), 10 mM acetate, 0.5 mM disodium EDTA). The denaturing gradient contained 35–70% denaturant (100% denaturant corresponded to 7 M urea and 40% formamide). Equal amounts of PCR products were applied to the DGGE gel. Electrophoresis was performed for 16 h at 75 V. The temperature was set at 60°C. DGGE gels were stained with ethidium bromide and photographed on a UV transillumination table with a CCD camera. The banding patterns were converted to a binary matrix and analyzed using the software program Bionumerics 5.1 (Applied Maths BVBA, Kortrijk, Belgium). Bands occupying the same position in the different lanes were identified and a pairwise similarity of the banding patterns of the different samples was calculated from band intensity in each lane (Pearson coefficient). Using these pairwise similarity values, a UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis was conducted to determine whether the samples reveal a non-random pattern, and whether they cluster according to incubation time or Daphnia density. For each experiment, only one start replicate of each treatment and both replicates of the final samples were analyzed. Per experiment, two parallel DGGE gels were run. To com-
Compare the banding patterns between the gels, we loaded a general marker sample and some of our experimental samples on both gels.

2.5. Statistical analysis

All statistical analyses on biovolume and abundance data were performed with the software PC program STATISTICA 5.1. [32]. Percentage biovolume of free rods and cocci was correlated to Daphnia dry weight using Spearman rank analysis. For the growth rates of the different microbial components, we first calculated polynomial regressions with Daphnia density. In case of a significant quadratic factor, piecewise linear regression was performed to find the breakpoint of the curve. When no quadratic factor was found, Spearman rank correlation analysis was done to elucidate the relation between growth rates and Daphnia dry weight [33].

3. Results

3.1. Experiment 1: Daphnia grazing on a bacterial assemblage pre-adapted to HNF predation

The microbial community, which had developed after removal of mesozooplankton, was characterized by high HNF densities and a high contribution of GRB morphotypes (Table 2). Incubating this community with different densities of Daphnia resulted in a top-down control of HNF and ciliates that clearly correlated with increasing Daphnia densities. In treatments with more than 30 Daphnia per liter (‘high Daphnia treatments’), the abundance of HNF was already strongly reduced after 24 h (Fig. 1a) and ciliates did not reach high densities during the incubation (Fig. 1b). In treatments with 20 or less Daphnia per liter (‘low Daphnia treatments’), however, two periods could be distinguished. During the first 24 h of the experiment, abundances of HNF remained relatively high (Fig. 1a). Then, ciliates developed (Fig. 1b), with oligotrichs and scuticociliates and to a lesser extent prostomatids as the dominant groups. When we calculated growth rates of HNF and ciliates only for the first 24 h of the incubation, we observed significant negative correlations with Daphnia abundance. Both correlations were quadratic functions with a breakpoint at an abundance of 30 Daphnia per liter (Fig. 2, Table 3). When we considered the whole incubation period of 67 h, no significant correlations between the growth rates of HNF or ciliates and the Daphnia abundance could be found (data not shown).

With respect to the bacterial community, total biovolumes of bean-like and dividing bacteria, free rods and cocci, aggregates and filaments also decreased rapidly and stayed low during the course of the experiment in the ‘high Daphnia treatments’ (Fig. 3). In treatments with 20 or less Daphnia per liter, total biovolumes of the different bacterial morphotypes remained high during the first 24 h of the incubation. Then, biovolumes decreased rapidly and reached levels similar to those in the ‘high Daphnia’ treatments (Fig. 3). Because the morphological distinction between the bacterial communities of the different bacterial morphotypes and HNF and ciliates at the start of experiment 1 (HNF-adapted bacterial community) and at the start of experiment 2 (Daphnia-adapted bacterial community)

<table>
<thead>
<tr>
<th>HNF-adapted community (ml⁻¹)</th>
<th>Daphnia-adapted community (ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free rods and cocci</td>
<td>9 ± 4·10⁶</td>
</tr>
<tr>
<td>Bean-like bacteria</td>
<td>2.4 ± 0.510³</td>
</tr>
<tr>
<td>Dividing bacteria</td>
<td>0.7 ± 0.310³</td>
</tr>
<tr>
<td>Filaments</td>
<td>16 ± 0.610⁴</td>
</tr>
<tr>
<td>Aggregates</td>
<td>19 ± 0.510³</td>
</tr>
<tr>
<td>HNF</td>
<td>10 ± 0.710³</td>
</tr>
<tr>
<td>Ciliates</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1 ± 0.7·10³</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 8·10⁶</td>
</tr>
<tr>
<td></td>
<td>2.8 ± 6·10⁶</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 0.7·10⁴</td>
</tr>
<tr>
<td></td>
<td>3 ± 0.9·10³</td>
</tr>
<tr>
<td></td>
<td>2 ± 0.8·10³</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>

Fig. 1. (a) Average HNF density (mean coefficient of variation (C.V.) = 0.55) and (b) average ciliate density (mean C.V. = 0.18) in the different treatments during an incubation period of 67 h in experiment 1.

Fig. 2. Quadratic regression of (24 h) growth rate of HNF (k = -0.01 + 0.001x - 8.10⁻⁵x²) and ciliates (k = 0.1 + 1.10⁻³x - 4.10⁻⁵x²) versus Daphnia abundance in experiment 1.

Fig. 3. (a) Average HNF density (mean coe⁄cient of variation (C.V.) = 0.55) and (b) average ciliate density (mean C.V. = 0.18) in the different treatments during an incubation period of 67 h in experiment 1.
**Table 3**

Quadratic regression statistics and piecewise linear regression data + breakpoint for growth rates of HNF and ciliates after 24 h incubation versus *Daphnia* density in the first experiment

<table>
<thead>
<tr>
<th>Piecewise linear regression</th>
<th>B</th>
<th>St. Err. of B</th>
<th>P-level</th>
<th>Breakpoint (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k HNF intercept</td>
<td>-0.011</td>
<td>0.021</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>k Daphnia</td>
<td>0.001</td>
<td>0.002</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>k Daphnia²</td>
<td>0.00001</td>
<td>0.00003</td>
<td>0.013</td>
<td>-0.071</td>
</tr>
<tr>
<td>k ciliates intercept</td>
<td>0.13</td>
<td>0.009</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>k Daphnia</td>
<td>0.0001</td>
<td>0.0009</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>k Daphnia²</td>
<td>-0.00004</td>
<td>0.00001</td>
<td>0.017</td>
<td>0.097</td>
</tr>
</tbody>
</table>

**3.2. Experiment 2: impact of *Daphnia* on a bacterial assemblage adapted to *Daphnia* grazing**

In experiment 2, the bacterial community was taken directly from the lake where it was already preconditioned to high *Daphnia* grazing pressure. It was characterized by lower abundances of larger bacterial morphotypes (bead-like and dividing bacteria, filaments and aggregates), and the predominance of small-sized rods and cocci (Table 2). Ciliates were almost absent and HNF occurred at low densities (Table 2). After exposing this microbial assemblage to the *Daphnia* gradient, significant changes in the bacterial community did not occur before 59 h of incubation. HNF concentrations increased modestly in the treatments with lower *Daphnia* densities after 48 h. In the zero *Daphnia* treatment, HNF increased to high levels above $3 \times 10^4$ ml$^{-1}$ at the end of the incubation (Fig. 7). Ciliate abundances remained very low during the course of the experiment ($< 10$ cells ml$^{-1}$, data not shown). Bacterial filaments increased only in the zero *Daphnia* treatment at the end of the incubation whereas bacterial aggregates developed modestly in all treatments with $< 20$ *Daphnia* l$^{-1}$ (Fig. 7). The biovolume of free rods and cocci after 59 h incubation, expressed as percentage of biovolume of the zero *Daphnia* treatment, was negatively correlated to *Daphnia* dry weight (Fig. 8). Growth rates of the different bacterial morphotypes were calculated for the whole 59-h incubation period. The growth rates of dividing cells, short and medium filaments and small- and medium-sized aggregates decreased significantly with increasing *Daphnia* dry weight per treatment (Fig. 9). We could not correlate growth rates of long filaments and large-sized aggregates to *Daphnia* density because they were absent in most of the treatments.

The DGGE fingerprint of experiment 2 showed marked differences in the composition of the bacterial assemblage between start and final samples and among *Daphnia* treatments (Fig. 10). During incubation, certain bands appeared or increased in relative intensity, while other bands disappeared or decreased in relative intensity. The observed changes differed between *Daphnia* treatments. UPGMA clustering based on presence–absence data of the bands resulted in a dendrogram with two distinct groups (Fig. 10). Samples of the initial bacterial community clustered together for all treatments and were clearly separated from the final samples. Moreover, we could distinguish between the final bacterial community in treatments with zero *Daphnia* per liter, treatments with 6–10 *Daphnia* per liter, and treatments with 20 or more *Daphnia* per liter.
4. Discussion

Our experiments reveal an important structuring role of filter-feeding Daphnia on microbial communities. In contrast to previous experiments, in which only treatments with and without zooplankton were compared, our design enabled us to assess the impact as a function of Daphnia density or dry weight. Overall, we can explain the observed patterns by the following scenario. As D. magna is a keystone predator [34,35] that feeds efficiently on a wide range of food types and sizes [36], all microbial components in our experiments are vulnerable to Daphnia ingestion [25]. The realized grazing impact on bacteria depends on the Daphnia concentration in relation to bacterial and protozoan production. In our experimental system, 30 Daphnia per liter was sufficient to efficiently control HNF and ciliates, resulting in Daphnia as the only significant bacterial consumer. When Daphnia abundance was lower than 30 individuals per liter, protozoa can sustain or develop and, at least partly, control the bacterial community. During the first 24 h of experiment 1, HNF were the only protozoan grazers that could contribute to shaping the bacterial assemblage because they had already reached high abundances due to their shorter generation times compared to ciliates. After 24 h, the appearance of oligotrichous ciliates belonging to the genus Halteria, scuticociliates and prostomatids, together with the presence of Daphnia, might explain the observed decline of HNF [37]. Oligotrichs feed efficiently on pico- and nanoplanckton [24,38]. Therefore ciliates may have become important bacterivores in treatments with 20 or less Daphnia per liter towards the end of the experiment.

Prey size is an important factor determining susceptibility to grazing [11,19,21,39]. Ciliates and Daphnia are filter feeders with the mesh width of the filtration apparatus determining the particle retention efficiency. Both bacterial consumers were, when present, able to suppress all bacterial morphotypes except the smallest freely suspended cells. Very small particles are also not efficiently ingested by most HNF [40,41]. This may explain the observed persistence and even increase of small unattached planktonic bacteria (rods and cocci) while larger dividing cells decreased under high HNF grazing pressure. Large beanlike bacteria must have developed other mechanisms that

![Graphs showing biovolume changes over time](https://example.com/graph.png)

**Fig. 3.** Biovolume of rods and cocci, bean-like and dividing bacteria, filaments and aggregates per ml in the different Daphnia treatments at each sampling time in experiment 1.

![Graph showing biovolume versus Daphnia density](https://example.com/graph.png)

**Fig. 4.** Biovolume of rods and cocci (as percentage of their biovolume in the zero Daphnia treatment, averaged over replicates per treatment, ±S.D.), versus average Daphnia dry weight (±S.D.) after 24 h incubation in experiment 1. Spearman $R = -0.79$ and $P = 0.0004$. 

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**H. Degans et al./FEMS Microbiology Ecology 42 (2002) 137–149**

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protect them against HNF grazing as they appeared in high densities under high HNF abundance. In chemostat experiments with a mixed freshwater bacterial assemblage, it was observed that bacteria with a similar type of morphology could achieve very high motility which made them less vulnerable towards HNF due to low capture efficiency (Matz et al. in prep.).

Large dimensions are also known to reduce sensitivity to grazing [42]. The majority of HNF feed on suspended bacteria and are limited by their own cell size in the ingestion of prey. As a result, the increase in size by the formation of filaments and aggregates under high HNF densities, as observed in our experiments, is an effective defence mechanism against HNF grazing [22,39,43,44]. On the other hand, the grazing efficiencies of the ciliate community and of Daphnia spp. are less sensitive to increasing prey size. Many ciliates have adopted many specialized feeding strategies [45] which enable them to cover as a group a large range of prey sizes. Daphnia spp. can filter particles up to more than 50 μm [46]. We indeed observed that growth rates of filaments and aggregates were negatively correlated with Daphnia density during the first 24 h of experiment 1. These morphotypes, while well protected against HNF predation, proved more vulnerable to Daphnia grazing. Moreover, after the appearance of ciliates, a shift towards a community with morphological characteristics similar to the bacterial communities in the treatments with 30 or more Daphnia per liter also occurred in treatments with 20 or less Daphnia per liter.

Our results are fully compatible with the results mentioned in literature [17,18,26] in that size-selective grazing by HNF can drive the community towards a high abundance of morphologically adapted, complex bacterial growth forms. The introduction of the keystone predator D. magna resulted in the suppression of HNF grazing. Due to differences in grazing selectivity between Daphnia and HNF, HNF-adapted bacterial morphotypes were efficiently grazed upon by Daphnia and an opposite shift in bacterial size distribution occurred. This was confirmed by our observation that growth rates of the different grazing-
GRB in the HNF-shaped bacterial community were negative in most Daphnia treatments, meaning that mortality was predominant in this experiment. In contrast, growth rates of GRB in the Daphnia-shaped bacterial community (exp. 2) were mainly positive. Moreover, the reduction in total biovolume of rods and cocci in the HNF-shaped bacterial community (exp. 1) already occurred under low Daphnia biomass and after 24 h incubation, while no clear reduction in biovolume of rods and cocci could be observed for the Daphnia-shaped bacterial community (exp. 2) under low Daphnia biomass, not even after 59 h incubation. Pre-adaptation to Daphnia grazing pressure thus results in a bacterial community less sensitive to Daphnia, and this holds also for the so-called morphologically vul-

Fig. 6. DGGE fingerprint and UPGMA dendrogram comparing initial and final bacterial assemblages in all treatments in experiment 1. Samples loaded on different gels are indicated with * and ‡.
It remains to be shown whether special survival mechanisms of bacteria against *Daphnia* grazing, such as viable gut passage, are widespread [47].

Our results clearly show the reversibility of morphological succession in the bacterioplankton community. An originally *Daphnia*-shaped bacterial community developed rapidly towards a HNF-adapted bacterial community after the release of *Daphnia* grazing pressure. This community returned towards the original situation in less than 24 h when high *Daphnia* densities were introduced again. Not only the introduction of *Daphnia* but also the release of *Daphnia* predation pressure resulted in pronounced changes in the bacterial community, but the rate at which the latter occurred was lower (59 h).

DGGE analysis of the bacterial communities in the different treatments does not provide clear-cut quantitative information of the bacterial community composition. However, this method may give us some background data on how bacterial communities may change under
different grazer conditions. The DGGE analysis indicated that the bacterial communities in our experiment not only respond phenotypically to grazing. Different grazers may drive the taxonomical composition of a bacterial community in distinct directions and do so in a short time span [16,17,23]. In experiment 2, differences in morphological characteristics between low and high Daphnia treatments at the end of the incubation were accompanied with differences in the DGGE profile. Interestingly, at the end of experiment 1, there was a discrepancy between the morphological and DGGE generated data: while no clear distinction could be made between low and high Daphnia treatments in terms of morphological characteristics of the bacterial assemblage, differences in the fingerprint data of the bacterial community were observed in the DGGE clustering pattern.

Previous studies have shown that morphological and compositional shifts in bacterial communities were related to substrate supply and bacterial growth rates [20,48,49]. Consequently, one may argue that the differential responses between high and low Daphnia treatments in our experiments could also have been the result of differences in bacterial substrate supply by grazers [50]. However, as the experiments were performed with water from eutrophic pond Lebrader Teich, we expect nutrient supply to be sufficient in all treatments to allow complex bacterial growth forms while the contribution of nutrient regeneration by grazers is expected to be of minor importance in our experiments. Moreover, the patterns we observed are reversed to what one would expect if nutrient regeneration by Daphnia had regulated bacterial density and growth.

Summarizing, our results clearly show a strong and differential impact of Daphnia on bacterial communities. The results of both our experiments revealed a negative relation of bacterial growth rates with Daphnia density, but absolute growth rates differed substantially (from net
growth to net losses) depending on whether the bacterial community had been shaped by high Daphnia densities prior to the experiment or not. The impact on bacterial growth rates was accompanied by pronounced and reciprocal changes in morphology. Moreover, our DGGE analyses indicated that part of these morphological changes are not purely phenotypically but could be associated with changes in taxon composition of the bacterial com-

Fig. 10. DGGE fingerprint and UPGMA dendrogram comparing initial and final bacterial assemblages in all Daphnia treatments in experiment 2. Samples loaded on different gels are indicated with ° and †.
munity. Our results also stress the importance of *Daphnia* in regulating aquatic microbial communities. In lakes where large cladocerans like *Daphnia* are virtually absent, direct grazing on bacteria mainly occurs by protozoans. In this case, bacterial communities are shaped by protozoan grazing and bacterial carbon is inefficiently transferred to higher trophic levels. However, even under moderate densities of 20–30 individuals per liter, *Daphnia* may already be the dominant bacterivore of the system through the elimination of protozoans. Moreover, in clear-water lakes or during a clear-water phase, *Daphnia* may attain very high densities (sometimes up to 2000 individuals per liter; [13]). In this case, *Daphnia* strongly controls all lower trophic levels including protozoans and bacteria and bacterial carbon is efficiently linked to higher trophic levels of the aquatic food web.

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