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MiniReview

Grazing of protozoa and its effect on populations of aquatic bacteria

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Abstract

Predation by bacterivorous protists in aquatic habitats can influence the morphological structure, taxonomic composition and physiological status of bacterial communities. The protistan grazing can result in bacterial responses at the community and the species level. At the community level, grazing-induced morphological shifts have been observed, which were directed towards either larger or smaller bacterial sizes or in both directions. Morphological changes have been accompanied by changes in taxonomic community structure and bacterial activity. Responses at the species level vary from species to species. Some taxa have shown a pronounced morphological plasticity and demonstrated complete or partial shifts in size distribution to larger growth forms (filaments, microcolonies). However, other taxa with weak plasticity have shown no ability to reduce grazing mortality through changes in size. The impact of protistan grazing on bacterial communities is based on the complex interplay of several parameters. These include grazing selectivity (by size and other features), differences in sensitivity of bacterial species to grazing, differences in responses of single bacterial populations to grazing (size and physiology), as well as the direct and indirect influence of grazing on bacterial growth conditions (substrate supply) and bacterial competition (elimination of competitors).

Keywords: Bacterium; Protist; Grazing on bacteria; Predation defence; Community structure

1. Introduction

Grazing by phagotrophic protists, especially bacterivorous nanoflagellates, has been identified as a significant factor modifying bacterial populations in aquatic ecosystems (e.g. [1]). Several studies have revealed grazing by protists as the dominant factor controlling bacterial mortality. However, in some habitats and seasons, metazoan grazing (e.g. by daphnids), or lysis by phages, may play a more important role [2–4]. Recent investigations have revealed that protistan grazing impacts both the bacterial standing stock and the morphological and taxonomic structure of bacterial communities [5].

In this review, we draw attention to factors that influence the effect of protistan predation on the morphology, composition and physiological status of bacterial cells in natural communities. We concentrate on both the influence of grazing on bacterial communities (consisting of several species) and on single populations. The review focuses on pelagic bacteria and on bacteria investigated in experimental systems simulating pelagic habitats. Metazoan grazing, lysis by phages, and the influences of physical and chemical factors, such as temperature, substrate availability, and nutrient supply, are not considered.

2. Selective grazing of bacterivorous protists and grazing-resistant bacteria

In general, grazing by bacterivorous protists upon suspended bacteria is selective. First of all grazing is size-selective [6–8] with most protists grazing preferentially on medium-sized bacterial cells. Protistan grazing on smaller and larger bacterial cells is less efficient. Bacteria which exceed in size the upper species-specific uptake limit of the predator are protected from grazing. On the other hand, despite the decrease of the uptake efficiency with particle size, no lower uptake limit exists. Some phagotrophic flagellates are even able to feed on virus particles [9] or high molecular weight polysaccharide [10]. Thus, the smallest bacterial cells are not completely protected from grazing but receive, due to lower grazing efficiency in com-

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parison to larger cells, a relative protection. Pernthaler et al. [11] have suggested functional size fractions within bacterial communities: small cells (< 0.4 μm), weakly affected by protists grazing, medium-sized 'grazing-vulnerable' (0.4–1.6 μm) and 'grazing-suppressed' (1.6–2.4 μm) bacteria, and large 'grazing-resistant' bacteria (> 2.4 μm).

Besides size, other traits of bacteria such as motility, shape, and cell surface characteristics may influence the selectivity of protistan grazing (e.g. [12]). Furthermore, it is known that the physiological state of the predators markedly influences the selectivity [13] and also species-specific selectivity of predators is likely [14,15]. Altogether, grazing by protistan predators on bacterial populations is a very complex interaction. The grazing efficiency and the vulnerability of the prey depends on a variety of factors.

Güde and Jürgens have suggested the term 'grazing-resistant bacteria' for bacteria with reduced vulnerability or a complete protection against protistan grazing [16,17]. Up to now, a precise definition or use of this term is difficult for the following reasons.

Firstly, besides the speculations of Jürgens and Güde [17], knowledge on size-independent grazing-resistant mechanisms of bacteria is still very scarce. To our best knowledge, the current literature provides no example of a size-independent grazing-resistance mechanism for a non-pathogenic bacterium. Features like motility, shape and surface characteristics may influence the grazing efficiency, but there is currently no evidence that such features may efficiently protect bacterial cells from grazing. Thus it remains unknown whether all bacterial cells in the edible size range for flagellates and ciliates are actually sensitive to protistan grazing.

Secondly, some bacteria may show an absolute resistance against protistan grazers (e.g. very large bacterial filaments, microcolonies or flocs). Others may only have a reduced vulnerability (e.g. small single-celled bacteria), and thus only obtain a relative 'resistance'.

Thirdly, grazing resistance of a particular bacterial strain may be restricted to one type of grazer or to a functional group of grazers. For example, short bacterial filaments in the size range of 4–10 μm would not be grazed by the majority of bacterivorous nanoflagellates, but could be sensitive to predation by larger flagellates or bacterivorous ciliates.

Fourthly, four bacterial strains grew in the presence of a bacterivorous flagellate as populations which were composed simultaneously of grazing-resistant and non-resistant cells [18–20]. These two sub-populations may have performed a dynamic exchange of individuals due to cell elongation and division.

Fifthly, differences in size-selective grazing are known for heterotrophic flagellates belonging to different taxa [8], and a potential influence of the predator's physiological status on the prey selectivity [13] has been demonstrated. Additionally, when considering only one predator species, the latter makes it very difficult to draw a line between grazing-resistant and grazing-sensitive bacteria in a given bacterial community.

Currently the terms 'grazing-resistant bacteria' and 'grazing-protected bacteria' are used in the literature for bacteria which are thought to be protected against protistan grazing due to their large size (filaments, other large complex growth forms and bacterial aggregates). Several size limits (e.g. > 2.0, > 2.4, > 2.5, > 3, > 4, > 5, and > 10 μm) have been used to separate these 'grazing-resistant' from the grazing-sensitive bacteria [21–26].

3. Impact of protistan grazing on cell morphology of single bacterial populations (species level)

Due to insufficient specificity of microscopic detection methods, no data on the influence of grazing on the morphology of single bacterial species are available from environmental studies. However, several batch and chemostat experiments have demonstrated that protistan grazing can impact the size distribution of populations of bacterial species [16,18–20,27,28]. Five species investigated responded to flagellate or ciliate grazing with a partial shift in size distribution towards large grazing-protected growth forms (filaments or suspended microcolonies). In the absence of predators, the bacteria grew in the edible size range of the predators and completely lacked larger (grazing-resistant) morphotypes. The predator-induced changes in morphology of each species resulted in populations consisting of both grazing-resistant and grazing-sensitive bacteria. Thus, the shifts in bacterial size distribution were the result of the increase in size of bacterial cells rather than changes in the proportion of small and larger bacteria. The response of predated populations to the experimental elimination of predation has been investigated for three out of the five species (*Flectobacillus major, Pseudomonas* sp. MWH1, and an unidentified strain) [19,27,28]. Size distribution of two species (*Pseudomonas* sp. MWH1, *F. major*) tended to shift back to the distribution observed before the introduction of the predator, but in one species the morphology developed under grazing pressure remained stable [28]. Loss of grazing-resistant morphology under predator-free growth was also observed by Güde in experiments with several bacterial species [16]. However, only a minority of bacterial strains appear to possess grazing defence strategies which are based on such morphological shifts ([27], Hahn, unpublished data).

Morphological shifts towards smaller cell sizes should also be an efficient defence strategy against protistan grazing, because decrease in particle size results in decrease of grazing efficiency. However, thus far, such a response of a bacterial strain or species to protistan grazing has not been described. On the other hand, grazing-induced shifts in mixed bacterial communities to smaller cell sizes were observed in experimental systems [15,27]. In one of these experiments, it was demonstrated that the morphological

Table 1
Observed effects of protistan grazing on bacterial communities and single bacterial populations

Observed effect of protistan grazing on bacterial communities/populations	Type of experiment	Remark	Reference
Change of morphological structure of bacterial communities:			
Freshwater bacterial community	Lab., chemostat	Bi-directional change towards larger and smaller types	[16]
Freshwater bacterial community	Field, mesocosms	Occurrence of bacterial filaments and aggregates	[22]
Marine bacterial community	Lab., mesocosms	Only three bacterial size classes were distinguished	[35]
Grazing-induced shift towards filamentous growth of bacterial species:			
Permanent shift to filamentous growth	Lab., batch cultures	Unidentified strain	[28]
Non-permanent shift to filamentous growth	Lab., chemostat	*Flectobacillus* spp., *Comamonas acidovorans*	[19]
Lost of filamentous growth after release from predation	Lab., chemostat	Unidentified strains	[16]
Control of bacterial filament formation by growth rate	Lab., chemostat	*Flectobacillus* spp., *C. acidovorans*	[19]
Grazing-induced formation of suspended microcolonies	Lab., chemostat	*Pseudomonas* sp.	[20]
Taxonomic shifts in bacterial community structure:			
Freshwater bacterial community[a]	Lab., continuous culture	In situ hybridisation with oligonucleotide probes (FISH)	[24]
Freshwater bacterial community[a]	Field, dialysis bags	In situ hybridisation with oligonucleotide probes (FISH)	[5]
Marine bacterial community	Field, bottle incubation	LH-PCR	[40]
Impact of size selectivity of grazing on both the morphological and the taxonomic structure of a bacterial community	Lab., chemostat	Species-specific bacterial size data	[27]
Involvement of both replacement and morphological adaptations of populations in response of bacterial communities to grazing	Lab., chemostat	Replacement of species and morphological adaptation of other species (morphological plasticity)	[27]
Impact of relief of predated communities from grazing on community structure of bacterial communities	Field, dialysis bags	Changes of bacterial community structure after release from predation	[5]
Change in bacterial activity pattern at the community level	Lab., continuous culture	Uptake of [^{3}H]thymidine and [^{14}C]leucine	[15]
Change in bacterial activity per cell at the species level	Lab., chemostat	Stimulation of nitrification by *Nitrosomonas europaea*	[43]
Predator-specific impact on morphological structure and bacterial activity pattern of bacterial communities	Lab., continuous culture	Influence of three predator species (*Bodo saltans*, *Cyclidium glaucoma*, *Ochromonas* sp.) was tested	[15]
Bacterial cell inactivation as response to protistan grazing (marine bacteria)	Field, dialysis bags	No direct evidence for cell inactivation	[47]

[a] Study demonstrated both impact of grazing on the taxonomic as well as on the morphological structure of the investigated bacterial community.

shift was a result of replacement of species with larger cells by smaller sized species [27].

Mechanisms controlling predation-induced changes in the morphology of single species have only been investigated for a few bacterial species [18–20]. Three species which responded to flagellate predation by filament formation responded in the absence of predators to increases in growth rates with the same morphological shifts [19]. However, a *Pseudomonas* strain which reacted to flagellate grazing with formation of grazing-protected suspended microcolonies did not react to increase in growth rate with similar morphological changes [20]. It has been suggested that inducible morphological grazing defence mechanisms of bacteria (e.g. filament formation) are triggered by chemical cues released by the protistan predators [15,29]. Yet, no evidence for this hypothesis has been presented.

Due to methodical difficulties it remains unknown whether bacterial phenotypic plasticity plays a significant role in the frequently observed grazing-induced occurrence of filamentous bacteria in natural bacterial communities. However, the observed rapid occurrence of high numbers of bacterial filaments within a few days after experimentally induced increase of protistan grazing [5,22,25] could be explained by phenotypic plasticity of bacteria, rather than by numerical increase of rare (below detection limit) permanently filamentous species.

4. Impact of protistan grazing on the morphological structure of bacterial communities (community level)

The first study which demonstrated the impact of bacterivorous protists on the morphological structure of bacterial communities has been performed by Güde in 1979 [16] (Table 1). He observed, in several continuous culture experiments with bacterial communities enriched from activated sludge, that increasing grazing activity by flagellates shifted the morphological structure of the bacterial community, from those consisting nearly exclusively of single-celled rods, to being dominated by large spiral-shaped and filamentous bacteria ('grazing-resistant bacteria'). He assumed that bacterial morphology and size strongly influenced the sensitivity of bacterial cells to flagellate grazing, and concluded that grazing by protists can shape the morphological structure of bacterial communities. During the following two decades, several publications have demonstrated the temporal or permanent presence or dominance of such grazing-resistant bacteria in numerous freshwater habitats [2,21,23,30–33]. These bacteria represented a broad range of complex morphotypes. Most of the reports have considered filamentous bacteria as the most abundant grazing-resistant morphotypes (e.g. [23,30]). The term filamentous bacteria covers elongated bacterial morphotypes of various morphology and taxonomy. The term has been used for linear and curved thread-like bacteria (no visible septae), as well as for chain-forming bacteria (for photos see [18,19,23,25]). It has been demonstrated that filamentous bacteria can belong to the phylogenetic groups of α- and β-subclass of *Proteobacteria* as well as to the *Cytophaga–Flavobacteria* group [5,18, 19,25]. Besides the filamentous bacteria, other complex bacterial morphotypes have been described, such as spiral-shaped and star-like cells or bacterial flocs, clumps and aggregates (e.g. [2,33]). Among the numerous publications which have reported the presence of grazing-resistant morphotypes in bacterioplankton, only a few descriptive field studies have demonstrated a correlation of high protistan grazing pressure and occurrence of morphologically defined grazing-resistant bacteria [11,30,31]. Several laboratory studies, however, have demonstrated that filamentous and other complex morphologies provide efficient protection against protistan grazing (e.g. [16,19,24,28]). Filamentous morphology may provide other advantages. Weinbauer and Höfle [34] found, in an investigation of bacterioplankton in the oxic layer of Lake Plußsee, that viral lysis was size-specific and that bacteria larger than 2.4 μm were not infected with viruses. In several acidified lakes in Bohemian Forest, that were free of zooplankton, the numerical dominance of filamentous bacteria in bacterioplankton has been observed over long periods, and exhibited no clear relationship to protistan grazing pressure (Vrba and Nedoma, personal communication; Nedoma et al., submitted for publication).

Experimental field studies have demonstrated that natural bacterial communities could respond within 1–2 days to experimentally enhanced protistan grazing pressure with tremendous shifts in the morphological structure [5,22,25]. Communities dominated by small and medium-sized single-celled bacteria shifted to communities dominated by filamentous and aggregated bacteria. Very similar responses have been observed in several laboratory studies with more or less complex bacterial communities cultured in different experimental systems [18,24,26,27,35]. A different response has been observed by Posch et al. [15]. A bacterial community cultured in three vessels of a flow-through system responded to grazing by three different protistan predators with no apparent shifts towards large protected morphotypes. In the presence of one predator species, grazing resulted in a pronounced shift towards small single-celled bacteria [15]. Posch et al. [15] explained the lack of larger bacterial morphotypes, such as filaments and aggregates, by a lack of species able to form such large morphotypes in the bacterial community studied.

Güde suggested in his review that intensive protistan grazing should lead to a bi-directional shift in size distribution of pelagic bacterial communities [32]. Grazing should shift size distribution towards large completely grazing-resistant morphotypes (e.g. filamentous bacteria) and simultaneously towards smaller single-celled bacteria which are, due to their small size, relatively protected against grazing. This hypothesis has recently been supported by experimental work demonstrating that a complex bacterial community cultured in a chemostat responded to the introduction of a bacterivorous flagellate with bi-directional shifts in the bacterial size distribution [27]. Elimination of the flagellate population resulted in a reversal towards the bacterial size distribution observed before the introduction [27]. On the other hand, several field and laboratory studies dealing with the influence of protistan grazing on the structure of bacterial communities reported no clear bi-directional shifts but mono-directional shifts towards larger or smaller bacterial morphotypes [15,18,22,24,25,35]. In the case of the laboratory investigations, however, bacterial communities might have lacked members able to form larger or smaller morphotypes [15]. Additionally, it is possible that in some studies bi-directional shifts have been overlooked due to insufficient resolution in analysis of bacterial size distribution data (e.g. due to too low numbers of size classes).

It is known that the size of bacteria is influenced by several other factors such as growth rate, temperature, oxic/anoxic conditions (e.g. [36]), and some studies have observed short-term changes in bacterial size distribution which were not correlated to changes in grazing pressure (e.g. [21,37]). These observed short-term changes in bacterial size distribution, however, have been much less pronounced than the observed short-term response of bacterial communities to intensive protistan grazing [5,22,25]. On the other hand, non-grazing effects on bacterial size might influence bacterial size distribution, thus masking bi-directional shifts induced by the grazers.

5. Impact of protistan grazing on the taxonomic structure of bacterial communities

The first study which demonstrated, by use of an in situ technique, that protistan grazing influences the taxonomic composition of bacterial communities was performed by Šimek et al. [24]. They used an experimental flow-through system to culture a mixed bacterial community and investigated, by in situ hybridisation with fluorescently labelled oligonucleotide probes, the impact of protistan grazing on the taxonomic composition of the bacterial community. This finding was supported by several other studies which

Table 2
Influence of protistan grazing on the taxonomic structure of bacterial communities as observed in various experimental systems

Bacterial community	Type of experiment	Method used for detection of compositional changes	Compositional changes (without/with protistan predation)	Simultaneous change of taxonomic and morphological structure	Reference
F, inoculum from activated sludge from sewage plant	Chemostat (dextran as substrate)	Cultivation on agar plates	Change of the colony composition	+	[16]
F, inoculum from algal culture	Continuous flow system (algal exudates as substrate)	FISH	Dominated by β-*Proteobacteria* (without); dominated by α-*Proteobacteria* (with)	+	[5]
F, mesotrophic Lake Constance	Chemostat (complex substrate)	Immunofluorescence microscopy	Dominated by *Vibrio* sp. (without); dominated by *Pseudomonas* sp. (with)	+	[27]
F, from small fishless pond	Bottle experiment	FISH	Complex group-specific succession after enhancement of protozoan grazing	+	[25]
F, mesotrophic Rimov Reservoir Clear water population (June)	Dialysis bags	FISH	Increase in α-*Proteobacteria* and *Cytophaga/Flavobacteria* group	+	[5]
Late summer situation	Dialysis bags	FISH	increase in α-*Proteobacteria* and *Cytophaga/Flavobacteria* group	+	
F, eutrophic Lake Loosdrecht	Continuous flow system	DGGE	Change in DGGE pattern	+	[26]
M, Mediterranean Sea	Mesocosms	DGGE	Change in DGGE pattern	+	[35]
M, Oregon coast	Bottle experiment	LH-PCR	Change in fragment size pattern	n.d.	[40]

F: freshwater, M: marine, FISH: fluorescent in situ hybridisation with group-specific rRNA-targeted oligonucleotide probes, DGGE: denaturing gradient gel electrophoresis of 16S rDNA amplicons, LH-PCR: length heterogeneity analysis after PCR of 16S rRNA genes.

used a variety of different methods, such as rRNA-targeted oligonucleotide probes [5,25], denaturing gradient gel electrophoresis (DGGE) [26,35], immunofluorescence microscopy [18,20,27], low molecular mass (LMW) RNA profiles [38,39] and length heterogeneity analysis by PCR (LH-PCR) [40] (Table 2). Investigations which focused on both the morphological and the taxonomic structure revealed that protistan grazing influences both. Whilst most studies demonstrated that occurrence or enhancement of grazing pressure on bacterial communities changes the taxonomic and morphological structure, two other studies demonstrated that the relief of predated communities from grazing can also influence taxonomic composition and morphological structure [5,27].

Field studies comparing grazing pressure and community structure of bacterioplankton are scarce. In an investigation of five mesotrophic to hypereutrophic lakes, a strong statistical relationship has been found between the composition of bacterioplankton communities (as determined by DGGE of 16S rDNA) and the biomass of potential protozoan grazers, i.e. ciliates and chrysophycean flagellates [41]. These results suggest that grazing pressure by protozoans can select for certain bacterioplankton taxa, at least in eutrophic lakes. For the eutrophic Lake Plußsee, it has been demonstrated that grazing by heterotrophic nanoflagellates accounted for 80–100% of the mortality in the upper epilimnion [3]. But even the high grazing pressure during the spring phytoplankton bloom, when heterotrophic flagellate numbers peaked, did not reduce the overall diversity of the bacterioplankton as determined by LMW RNA profiling [39]. By contrast, during the clear water phase following the phytoplankton spring bloom, overall diversity of the bacterioplankton was strongly reduced, but this reduction was due to metazoan grazing and not induced by protozoan grazing. This example has demonstrated, at least for a eutrophic situation, that bacterioplankton communities can have a high diversity despite high protistan grazing pressure as long as there are diverse and readily available carbon sources.

For oligotrophic marine systems, the community structure seems to be primarily controlled by the hydrography of the system, although Murray et al. [42] speculated that archaeal marine bacterioplankton could be controlled by grazing nanoflagellates.

6. Interplay of grazing-induced changes in morphological and taxonomic structure of bacterial communities

Most studies on the influence of protistan grazing on mixed bacterial communities have not used methods enabling detection of morphological and compositional changes at the species level. Thus it has not been possible to reveal a possible interplay of these two effects. However, one study demonstrated, by combined detection of specific bacterial numbers and measurement of specific bacterial cell size, that changes in bacterial size distribution were linked to changes in bacterial community com-

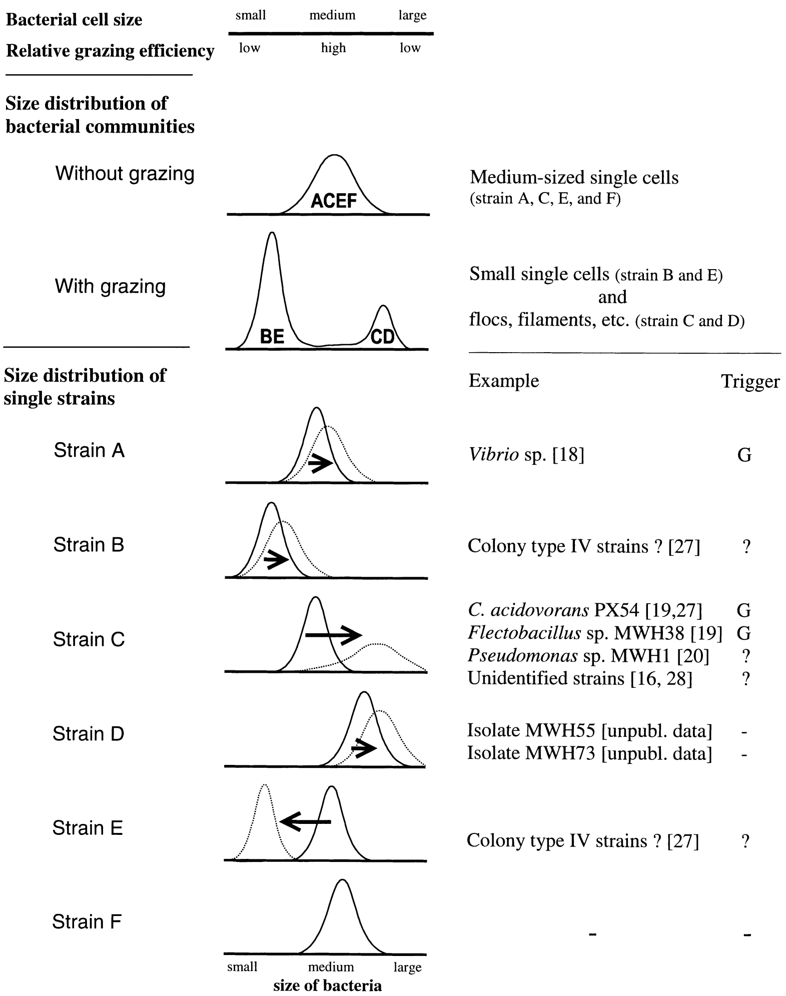

Fig. 1. Scheme summarizing the interplay of size-selective grazing, morphological structure of bacterial communities and strain populations as well as the taxonomic composition of bacterial communities. The scheme is based on results from several chemostat and batch culture experiments ([18–20,27], Hahn, unpublished data) and considers only size selectivity of protistan grazing. Grazing selectivity based on other prey features and size-independent bacterial defence strategies, as well as other parameters like bacterial competition, release of substrates and nutrients by the grazers, etc., are not considered. The upper part of the scheme depicts the size selectivity of protistan grazing. The middle part shows the size distribution of bacterial communities in situations with and without grazing, as well as the possible strain composition (indicated by letters). The lower part depicts the size distribution of six single bacterial strains (A–F), as well as grazing-influenced changes in their size distributions (dotted curves). The arrows indicate the direction and strength of grazing-induced changes in the size distribution of the strain populations. Examples for the size distributions shown and patterns of change are given, and the triggers (G = growth rate, ? = unknown) responsible for controlling the changes are indicated. Examples labelled with question marks provide assumed size distributions and grazing responses. Differences in the size distribution patterns and differences in responses to grazing by single bacterial strains result in different grazing sensitivity of the strains. This leads to grazing-induced changes in the morphological structure and the taxonomic composition of bacterial communities.

position [27]. Size-selective grazing by a bacterivorous flagellate decreased the number of dominating strains which possessed medium-sized cells. These strains were largely replaced by single-celled strains with smaller cells or by bacteria which formed large grazing-protected morphotypes (suspended microcolonies). Additionally, this experiment demonstrated that both replacement of species and adaptation due to phenotypic plasticity can be involved simultaneously in the grazing-induced changes of bacterial communities. For this experiment, however, it has been concluded that size-selective grazing was the major force controlling both the morphological and the taxonomic structure of the bacterial community [27]. In Fig. 1, a scheme is given depicting in a simplifying way the interplay of flagellate grazing and the response of single bacterial populations, as well as the response of the whole bacterial community.

7. Impact of protistan grazing on the physiological status of bacteria

Several studies demonstrated that protistan grazing enhances activity of bacterial species populations and mixed bacterial communities. Verhagen and Laanbroek [43] observed in chemostat studies with nitrifying bacteria that predation by a flagellate increased per cell bacterial activity. Šimek et al. [44] found a significant correlation between the total grazing rate of flagellates and the proportion of metabolically active bacteria (as determined by microautoradiography) for bacterioplankton inhabiting Rimov Reservoir. In an experimental system, Posch et al. [15] measured predation-induced changes in per cell uptake of [^{3}H]thymidine and [^{14}C]leucine. They observed that an identical bacterial community responded to predation by different predator species with different changes in the uptake of the two tracers. Whilst in the study of Verhagen and Laanbroek [43] the per cell activity of one bacterial population was enhanced, it is unknown whether the changes in the two latter studies were due to replacement of less active by more active bacterial populations. The same holds true for the observed enhancement of the bacterial breakdown of the polysaccharide cell wall of *Peridinium* cells by flagellate predation [45].

Several influences can be responsible for grazing-mediated increase in bacterial activity (see [15,27,43] for references and discussion). Firstly, nutrient-limited bacteria can profit directly from substrates released by the predators. Secondly, bacteria can profit indirectly from nutrients released by predators via enhancement of primary production of nutrient-limited algae [46]. Thirdly, predators may decrease the numbers of intra- and inter-species competitors and thus increase the supply of substrates per cell which should result in higher bacterial growth rates and a higher activity per cell or unit biomass. Fourthly, protozoan grazing may select for bacterial populations which are able to balance the loss rates with higher growth rates, resulting in a shift to bacteria with higher activity [29].

The opposite response of bacteria to strong grazing pressure has been hypothesised by del Giorgio et al. [47]. They suggested that bacteria of predated populations escape from grazing by cell inactivation. Inactive cells have been found to be smaller than active cells (e.g. [48]), and thus should receive a relative grazing protection due to size selectivity of protistan grazing. A study on a coastal bacterial community has demonstrated that cell inactivation combined with preferential grazing on active (larger) cells resulted in a grazing driven increase of the percentage of inactive bacterial cells [47].

However, it is not known whether bacteria from different habitats respond similarly at the physiological level to protistan grazing. Several ecological parameters might influence the physiological response of single bacterial populations and thus of the whole communities.

8. Influence of the trophy of the aquatic ecosystem on the response of bacterial communities to protistan grazing

The majority of studies which have dealt with the impact of grazing on the morphological and taxonomic structure of bacterial communities focused on meso- to eutrophic freshwater ecosystems, or have used experimental systems with conditions roughly comparable to these habitats. No study on an oligotrophic system and only a few studies on communities from coastal marine water have been performed [35,40]. Thus it is not known whether the observed strong impact of protistan grazing on morphological and taxonomic structure of bacterial communities is restricted to systems with higher trophy, or whether this also holds true for oligotrophic marine and freshwater systems. Probably, bacterial communities of oligotrophic systems are, in terms of impact on morphological and taxonomic structure, less sensitive to protistan grazing because of the stronger bottom-up control of these communities.

9. Future research directions

To gain deeper insights in the interplay of protistan grazing with the morphological and taxonomic structure of bacterial communities, more field studies focusing on both the protistan grazing and the structures of bacterial communities are needed, especially in oligotrophic environments. Additionally, field investigations are needed which link taxonomic data of a high resolution to morphological data. This could be provided by a combination of fluorescent in situ hybridisation (FISH) with species-specific oligonucleotide rRNA-targeted probes and image analysis [49]. Use of the combination of FISH and autoradiography [50] in grazing experiments will help to reveal the influence of grazing on the activity of different bacterial taxa. Furthermore, investigations on the influence of viruses and bottom-up control mechanisms on the interplay of bacteria and bacterivorous protists are needed.

Current knowledge on bacterial grazing defence strategies is more or less limited to size-dependent strategies such as filament and microcolony formation. Because protistan predators and bacterial prey coexisted over long geological periods, one can expect that a broad diversity of grazing defence strategies have evolved in bacterial populations. This may include size-independent strategies or post-ingestional strategies. Thus, for a deeper understanding of the bacteria–protozoa interplay, extension of the knowledge on the diversity of bacterial grazing defence strategies is needed.

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