

Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates

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ABSTRACT: The effect of suspended fine sediment on protist growth and behaviour was investigated using kaolinite-dominated clay with a mean grain size of 0.9 μm . Suspended sediment particles about the same size as potential food items of picovorous organisms, i.e. bacteria, were expected to interfere with feeding. Growth rates of *Halteria* sp. and *Tetrahymena pyriformis* were 0.056 and 0.15 h^{-1} irrespective of the clay concentration, *Cyclidium* sp. showed slightly higher but significant growth rates in the presence of clay, as did most flagellates. The largest difference in growth was found for *Spumella* sp., which showed rates of 0.19 and 0.14 h^{-1} with and without clay, respectively. An exception was the gliding flagellate *Entosiphon sulcatum*, which was covered by clay particles and therefore hardly came in contact with food particles. Flagellates discriminated strongly against sediment particles prior to food uptake. With decreasing particle concentration this selection mechanism became less important compared to differential digestion especially in *Spumella* sp. and an unidentified groundwater flagellate. Both *Bodo saltans* and *Monosiga ovata* showed differential digestion as well, but their importance in overall selectivity was low even at a low particle concentration. In contrast to flagellates, the ciliates collected many particles, including clay particles and bacteria, and incorporated them into 1 food vacuole. Discrimination against clay was of minor importance in ciliates, but a high feeding capacity seems to compensate for this low selectivity. In contrast to many zooplankton species, it can be concluded that protists are generally well adapted to environmental situations of high sediment load.

KEY WORDS: Microbial food web · Suspended matter · Feeding strategy · Bacterivory · HNF · Plankton · Runoff · Resuspension

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INTRODUCTION

Suspended sediments are ubiquitously distributed in freshwater ecosystems, causing significant environmental damage and economic costs (Clark et al. 1985). They are abundant in lowland streams and rivers (Ganges, Yellow River, e.g. G. X. Li et al. 1998, B. G. Li et al. 1999, Xu 2000) and of general importance in all freshwater systems after precipitation or flood events (e.g. Lenzi & Marchi 2000). Suspended sediments enter the water column mainly by resuspension and surface flow from terrestrial ecosystems (Walling 1990, Evans 1994, Booth et al. 2000, Steegen et al. 2000). Suspended sediment concentrations in lakes and reservoirs often dramatically increase after

flood events. Low annual mean concentrations of less than 10 mg l^{-1} may increase to more than 200 mg l^{-1} (Dokulil 1979, Knowlton & Jones 1995). In more turbid environments such as rivers, lake inflow and lakes with higher turbulence in general, even the mean suspended sediment concentrations are up to more than 100 mg l^{-1} (Arruda et al. 1983, Brondson & Naden 2000, Orton & Kineke 2001). Concentrations may increase temporarily by several orders of magnitude due to washout after heavy precipitation, flood events, and wind-induced resuspension (e.g. Brondson & Naden 2000, Lenzi & Marchi 2000). For subtropical lowland streams even concentrations of several g l^{-1} are the normal load and concentrations of up to 100 g l^{-1} are reported for peak events (e.g. Yellow River and

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Ganges: Velegrakis et al. 1997, B. G. Li et al. 1999, Xu 2000).

Grain size distribution of the suspended particles is dependent on flow velocity. Mean particle size is, therefore, highest in turbulent mountain streams and decreases for lowland rivers and lakes. Whereas particles of silt and sand fraction may dominate in streams, the importance of small particles of clay fraction increases for water bodies of low turbulence. Depending on the freshwater body, about 10 to >80% of the suspended sediment mass belongs to the clay fraction (<2 μm ; for an overview see Walling & Moorehead 1989). These fine suspended sediment particles may be several orders of magnitude more abundant than similar sized organisms, i.e. bacteria, protists and phytoplankton.

Suspended sediment load is directly linked to water clarity and light penetration into waters (Davies-Colley & Smith 2001). Consequently, a direct impact on primary production is expected and has long been recognised (Burkholder & Cuker 1991, Hellström 1991). Increased inorganic turbidity decreases light penetration and therefore may lead to a decrease of phytoplankton biomass and productivity (Grobbelaar 1985). As population growth rates of cladocerans and rotifers are often limited by food availability (Edmondson 1965, Tessier & Goulden 1982), a decrease of phytoplankton due to increasing turbidity may indirectly affect zooplankton populations.

With increasing suspended clay concentrations the feeding pressure of visual predators (e.g. fish) may decrease, leading indirectly to an increased population growth of prey organisms (e.g. bacterivorous plankton: (Chiasson 1993). Prey-sized suspended clay may also cause a reduced feeding efficiency, especially of filter-feeding organisms (e.g. cladocera: Arruda et al. 1983, Hart 1988, Kirk & Gilbert 1990, Kirk 1991). High abundances of particles in the size range of potential food particles leads to reduced population growth rates or even dying back of cladoceran populations (McCabe & O'Brien 1983, Kirk & Gilbert 1990). As rotifers are less susceptible to high abundances of suspended sediment particles, they may become dominant in lakes usually dominated by cladocerans when suspended sediments are introduced (Kirk & Gilbert 1990).

The significance of suspended sediments for bacterial production and the microbial loop is not yet fully understood. Bacteria have been shown to be associated with particles in clay rich waters and particle-attached organics have been suggested to support bacterial growth especially in nutrient poor waters where the bulk of dissolved organic matter is attached to particles (Fletcher & Marshall 1982, Lind et al. 1997). It has also been suggested that bacterial production might be decoupled from primary production as light,

not nutrients, may become limiting for primary producers (Lind et al. 1997). Interactions similar to those which occur in metazooplankton may also occur in bacterivorous protists. These organisms are confronted with high concentrations of indigestible particles in the size range of possible prey organisms (0 to 5 μm ; clay-silt). The time-consuming handling of such indigestible particles by protists (ingestion, egestion, etc.: (Boenigk et al. 2001) could influence protist population dynamics (England et al. 1993). Despite the central role of bacterivorous protists in aquatic ecology (Azam et al. 1983, Jumars et al. 1989) and the presumably high impact of suspended clay on protists (England et al. 1993, Boenigk et al. 2002) this aspect has not been investigated in detail. A notable exception is the study by Jack & Gilbert (1993), who reported a decreased growth rate in the presence of clay for the ciliate *Strobilidium* sp., but no effect on the ciliates *Bursaditium* sp., *Euplotes* sp. and *Paramecium* sp.

Protists possess a variety of selection mechanisms which may be important for discriminating against suspended sediment particles. Boenigk et al. (2002) suggested that active selection by protists may be an important mechanism for selecting between food and non-food objects, rather than between different bacterial strains. Suspended clay is in the size of potential food bacteria and, in turbid environments, is much more abundant than bacteria. We, therefore, tested the hypothesis that protists are negatively affected in their population growth rate when suspended sediments are introduced. We also qualitatively investigated the handling of clay particles by protists of contrasting feeding modes, as particle handling and food selection mechanisms were expected to be explanatory for the differences between species.

MATERIALS AND METHODS

Artificial media and clay. The clay originated from the clay pit Kärlich in the Eifel, Germany. The clay contained 84% kaolinite, 5% opal, 3% montmorillonite, 3.5% rutile, 2% quartz, and 2% goethite (Heide 1955). Organic matter content of the clay was 0.4% of the clay dry weight and therefore accounted for less than 1% of the total organic carbon in the experiments. Grain size spectrum of the original clay was scaled down by sedimentation. This treatment reduced the portion of larger sediment particles significantly (Fig. 1). A stock solution containing 10 g l⁻¹ clay was prepared and used for all of the experiments. Clay concentrations of 0, 50 and 200 mg l⁻¹ were used for the experiments. Particle abundance was counted after 4; 6-diamidino-2-phenylindole (DAPI) staining. Colloidal particles were present, but due to their minor contribu-

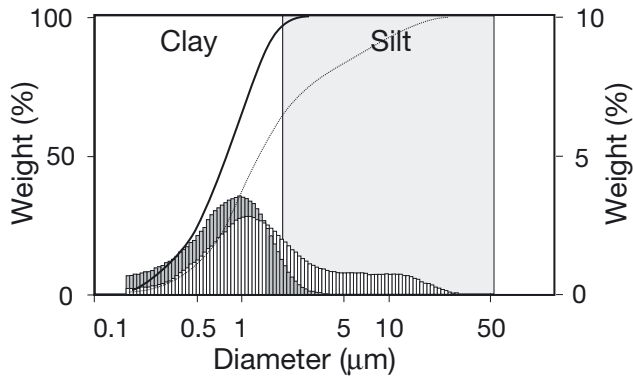


Fig. 1. Grain size distribution of the kaolin-rich clay used in the experiment. Grain-size distribution curve and sum curve are shown for the original clay as obtained from the clay pit (white bars and dashed line) and for the clay after the washing procedure to reduce the fraction of larger particle sizes (gray bars and black line). Note the logarithmic scale of the abscissa

tion to suspended sediment mass and the decreasing importance of smaller particles as 'bacterial equivalents' particles $<0.2 \mu\text{m}$ were not counted. The clay concentrations used in the experiment correspond to a particle abundance of $>5 \times 10^8$ particles ml^{-1} (50 mg l^{-1}) and $>2 \times 10^9$ particles ml^{-1} (200 mg ml^{-1}).

Standard medium for the experiments was Pratt medium. Pratt medium contains $0.1 \text{ g l}^{-1} \text{ KNO}_3$, $0.01 \text{ g l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.01 \text{ g l}^{-1} \text{ K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ and $0.001 \text{ g l}^{-1} \text{ FeCl}_3 \cdot 6\text{H}_2\text{O}$. For culturing bacteria, as well as for

the experiments (see 'Live observations' and 'Growth experiments') basic medium supplemented with nutrient broth, soyotone, peptone, and yeast extract (all obtained from Difco) were used (each 10 mg l^{-1}). Addition of organic complex media roughly corresponded to 5×10^7 bacteria ml^{-1} . For the maintenance of protist cultures, the inorganic basal medium (see above) supplemented with a wheat grain was used.

Organisms. We chose bacterivorous protists belonging to several taxonomic groups representative of lakes and rivers (see Table 1). The most common feeding types (filter- and interception feeding) and motile, as well as sessile, organisms were chosen. All protists were maintained in inorganic Pratt medium supplemented with a wheat grain in 50 ml tissue culture flasks (Sarstedt®). All organisms were kept in the dark at $15 \pm 0.5^\circ\text{C}$. Forty-eight h before the experiments started the protists were transferred to Erlenmeyer flasks (in the case of *Vorticella similis* a 200 ml tissue culture flask was used) containing enriched Pratt medium and cultured on a mixture of the background bacteria and *Pseudomonas putida* strain MM1 (isolated by Christoffersen et al. 1997).

Determination of cell numbers and cell size. Sub-samples were obtained from the experimental vessels after moderate shaking. Flagellate cell numbers were determined from 1 ml subsamples from live counts using a Sedgewick Rafter chamber at $200\times$ total magnification using an inverted microscope. All ciliates except *Vorticella similis* were counted accordingly

Table 1. Feeding type and origin of species used in the investigation. Cell volume was calculated by applying simple geometric equations based on measurements of cell length and width (see 'Determination of cell numbers and cell size')

Species	Taxonomic group	Cell volume (μm^3) Mean \pm SD	Dominant feeding type	Originated/isolated
<i>Spumella</i> sp.	Chryomonadida	63 ± 21	Interception	Lake Schoehsee by K. Jürgens
<i>Monosiga ovata</i>	Choanoflagellida	62 ± 11	Diffusion/Filter	Small pond near Rybinsk reservoir by A. P. Mylnikov
<i>Bodo saltans</i>	Kinetoplastida	74 ± 12	Interception	Small pond near Rybinsk reservoir by A. P. Mylnikov
<i>Entosiphon sulcatum</i>	Euglenida	1080 ± 208	Raptorial	CCAP 1220/1a
'Tony'	<i>Incertae sedis</i>	202 ± 54	Diffusion/Raptorial	Otis aquifer, MA, USA by G. Novarino
<i>Cyclidium</i> sp.	Ciliata	887 ± 294	Filter/Interception	Originally M. Macek, re-isolated by K. Šimek from Rimov reservoir
<i>Vorticella similis</i>	Ciliata	24020 ± 8160	Filter	CCAP 1690/2
<i>Halteria</i> sp.	Ciliata	10630 ± 2455	Filter	Small pond in Cologne by S. Wickham
<i>Tetrahymana pyriformis</i>	Ciliata	19340 ± 4770	Filter	Sciento®, Manchester, UK

using a 50× total magnification. *V. similis* attached heavily to the bottom of the experimental vessel. This species was, therefore, directly counted in the experimental vessels using binoculars. Cell volumes were calculated with live observations. Of each protist species, 20 to 50 cells were measured from a calibrated video screen and cell volumes were calculated assuming an ellipsoid cell shape (in the case of *V. similis* a cylinder with a terminal cone and in the case of *Bodo saltans* a cylinder with half-spheres at both ends was assumed).

Bacterial cell numbers were determined from formaldehyde (2%) fixed subsamples. They were stained with DAPI and counted by epifluorescence microscopy. Enumeration of bacteria in the presence of clay is generally problematic as the clay is also stained by fluorescent dyes even by so-called 'specific' probes. In contrast to natural samples of a high sediment load, e.g. soil samples, most sediment particles in our experiment were unflocculated single particles of approx 0.5 to 3 µm. In addition, bacterial numbers became quite low towards the end of the experiments. Strong dilution of the media reduced the problem of bacterial counts, but did not eliminate it altogether. For high concentrations of suspended fine sediments but low bacterial numbers there is still not yet a satisfactory solution. Although the development of bacteria could be roughly estimated during the experiments, absolute counts were only possible during the first subsamplings. For later times of the experiment when bacteria were grazed below approx. 10^7 bacteria ml⁻¹ no reliable counts were possible. Bacterial abundance was, however, between 2×10^7 and 5×10^7 ml⁻¹ during the initial phase of the experiment from which the protist growth was calculated. From previous experiments it can, therefore, be assumed that the flagellates were food satiated (Boenigk et al. 2002, author's unpubl. data). Due to the high suspended sediment concentrations and less selective feeding of the ciliates, satiating conditions can also be assumed for the ciliates at least for the treatments with suspended clay (Pfandl et al. unpubl.).

Live observations. High-resolution video microscopy was used for live observations of protist behaviour and feeding (15 replicates each). Feeding and swimming behaviour of protists was investigated using a total magnification of 800 to 2500×. The use of an inverted microscope equipped with 40 and 100× oil immersion objectives has proved to be suitable for the investigation of several flagellate and ciliate species (see (Boenigk & Arndt 2000)). For the observation of larger ciliates a 20× objective and for an overview a 5× objective were used. The microscope was equipped with an 1.6 and 2.5× optovar and a video zoom adapter. All observations were recorded on S-VHS tapes using a charge-coupled device (CCD) camera

(Model XC-ST70CE, Hamamatsu), which allowed us to enhance the digital contrast and analyse the specimen directly from the monitor.

All experiments were carried out at room temperature at moderate illumination. In previous experiments critical light levels which may cause artificial behaviour were checked. Extreme light conditions proved to be critical, especially for attached organisms (*Spumella* sp. and *Bodo saltans*), and seem most likely to be due to the heating of the bottom cover slide. In the experiment $\frac{1}{5}$ to $\frac{1}{10}$ or less of the critical light intensity was applied and therefore no light-induced behavioural changes were to be expected. *Vorticella similis* was transferred to the experimental vessels 24 h before the experiments to allow the organisms to attach to the bottom cover slide. All other protists were allowed to adapt to the experimental conditions for 30 min before the experiments started. Immediately before the observation started, clay suspension was added to achieve a final concentration of 50 mg l⁻¹. Due to sedimentation processes clay concentration and medium grain size both increased slightly during the observation time. This was, however, not critical for qualitative observations. Bacterial concentrations were around 10^7 ml⁻¹. Additional observations were carried out at strongly diluted conditions (up to 100-fold dilution) to check for concentration-dependent shifts in the feeding behaviour. For the flagellate species and *V. similis*, observations of individual cells were carried out for at least 15 min. If no egestion of clay particles was observed during this time, the observations were extended until egestion occurred. A new petri dish was then prepared. For details of the observation protocol see Boenigk & Arndt (2000).

For the observation of the highly motile ciliate species (*Tetrahymena pyriformis*, *Cyclidium* sp. and *Halteria* sp.) continuous observation of single cells was not possible. Therefore, the observation protocol was changed as follows: an individual cell was observed for 30 s. If the observed cell swam out of sight or after the 30 s, a new cell was chosen for observation. In this manner observations were carried out for 20 to 60 min (depending on the vacuole passage times of clay for the different species). A new petri dish was then prepared. This experiment was repeated until a permanent record of at least 15 individual cells for the entire time window was achieved. With this protocol information on individual variability was, of course, lost. The experimental protocol allowed, however, for the observation of the population mean behaviour.

Growth experiments. All experiments were carried out at 20°C and continuous illumination. The basic medium for the experiments was enriched Pratt medium. All experiments were carried out in 100 ml Erlenmeyer flasks except for *Vorticella similis*, for which 200 ml cell

culture flasks were used. Forty-eight h before the experiments were started, the organisms were adapted to the experimental conditions, i.e. satiating food concentration and experimental temperature and light conditions. At the start of the experiment bacterial concentration was adjusted to $2 \times 10^7 \text{ ml}^{-1}$ and the required clay concentration (0, 50 or 200 mg l^{-1}) was achieved by adding a suitable volume of clay stock solution. Initial abundance of *Spumella* sp., *Bodo saltans*, and *Monosiga ovata* was adjusted to 500 to 1000 flagellates ml^{-1} ; start abundance of *Entosiphon sulcatum* and 'Tony' was adjusted to approximately 5 to 10 flagellates ml^{-1} and start abundance of the ciliates was adjusted to 50 to 100 ind. ml^{-1} . In addition, controls containing only clay and bacteria were carried out. This protocol was only modified for *V. similis* as this ciliate had to adapt and attach to the experimental vessel for at least 24 h. Therefore, in the case of *V. similis*, experiments were carried out directly in the 200 ml cell tissue flasks. At the start of the experiment the medium was exchanged for fresh medium containing bacteria and clay. Each experiment was run in 3 replicates. The final volume at the start of the experiment was 50 ml and subsamples of 3 ml were taken after 0, 8, 24, 32, 48, 56 etc. h until the stationary growth phase was reached. The experimental vessels were shaken prior to sampling. Growth rate (G) was calculated from 24 h intervals from the exponential growth phase according to the formula

$$G = (\ln C_e - \ln C_s)/24 \quad (1)$$

where C_e is the abundance of protists at the end of the 24 h interval and C_s the abundance at the beginning. For the calculation the first 3 time points fitting to exponential growth (except 0 h) were used. The start abundance (0 h) was always ignored for the calculation even though it might fit to the linear curve, as some minimum time to adapt to the different clay concentrations had to be taken into account.

RESULTS

Growth experiments

The presence of clay had significant effects on the population growth rates and maximal abundance of the protists investigated here. In general, most species (except *Entosiphon sulcatum*) were only slightly negatively affected by the presence of clay, but in some cases the growth rate and maximal abundance showed an increase. *Bodo saltans*, *Spumella* sp. and 'Tony' showed significantly higher growth rates in the presence of clay (ANOVA; $p < 0.05$; see Fig. 2). *Spumella* sp. and *B. saltans* also showed increased maximal abundances in the presence of clay (ANOVA; $p <$

0.001 ; see Fig. 2), whereas in the presence of clay 'Tony' showed a decrease in maximal abundance. Both the growth rate and maximal abundance of *Entosiphon* sp. were strongly negatively affected by suspended clay (ANOVA; $p < 0.001$; see Fig. 2). In *Monosiga ovata* the growth was hardly affected by clay even though the differences proved to be significant. The maximal growth rate of *M. ovata* increased in the presence of 50 mg l^{-1} clay, but decreased significantly at 200 mg l^{-1} clay (Fig. 2). In general the ciliates were more negatively affected by the presence of clay, and some species were more affected than others. For instance, in the presence of clay *Cyclidium* sp. showed a significantly higher growth rate, but its maximal abundance was significantly lower (ANOVA; $p < 0.001$). Except for a slight increase in the maximal abundance of *Halteria* sp. and a decrease of the maximal abundance of *Tetrahymena pyriformis* in the presence of clay, the growth rates and maximal abundances of the other ciliates were not significantly affected by clay (Fig. 2).

Live observations

Even though bacterial concentrations were rather high (10^7 ml^{-1}) they were only a minor fraction of the total particles present. Total particle concentration including the sediment particles exceeded 10^8 and therefore conditions favouring strongly selective feeding (Boenigk et al. 2002) were met. In general all flagellates showed a selection behaviour similar to that described elsewhere for *Spumella* sp. (Boenigk et al. 2001, 2002). Selective discrimination of clay particles during particle handling (after capture and before ingestion) was observed for all flagellates in addition to differential digestion. The relative importance of the various selection mechanisms was observed to be species-specific (Fig. 3).

Spumella sp.

Selection before food uptake as well as differential digestion occurs. At low particle concentration non-selective feeding on clay and bacteria occurs. Differential digestion was consequently the most important active selection mechanism at the lower particle concentration even though rejection of clay particles had already occurred. At high particle concentrations differential particle handling became the dominant selection mechanism and hardly any clay particles were ingested. The mechanism of selection, therefore, shifts with particle concentration, but irrespective of particle concentration, handling is particle-specific and results in effective discrimination against clay particles.

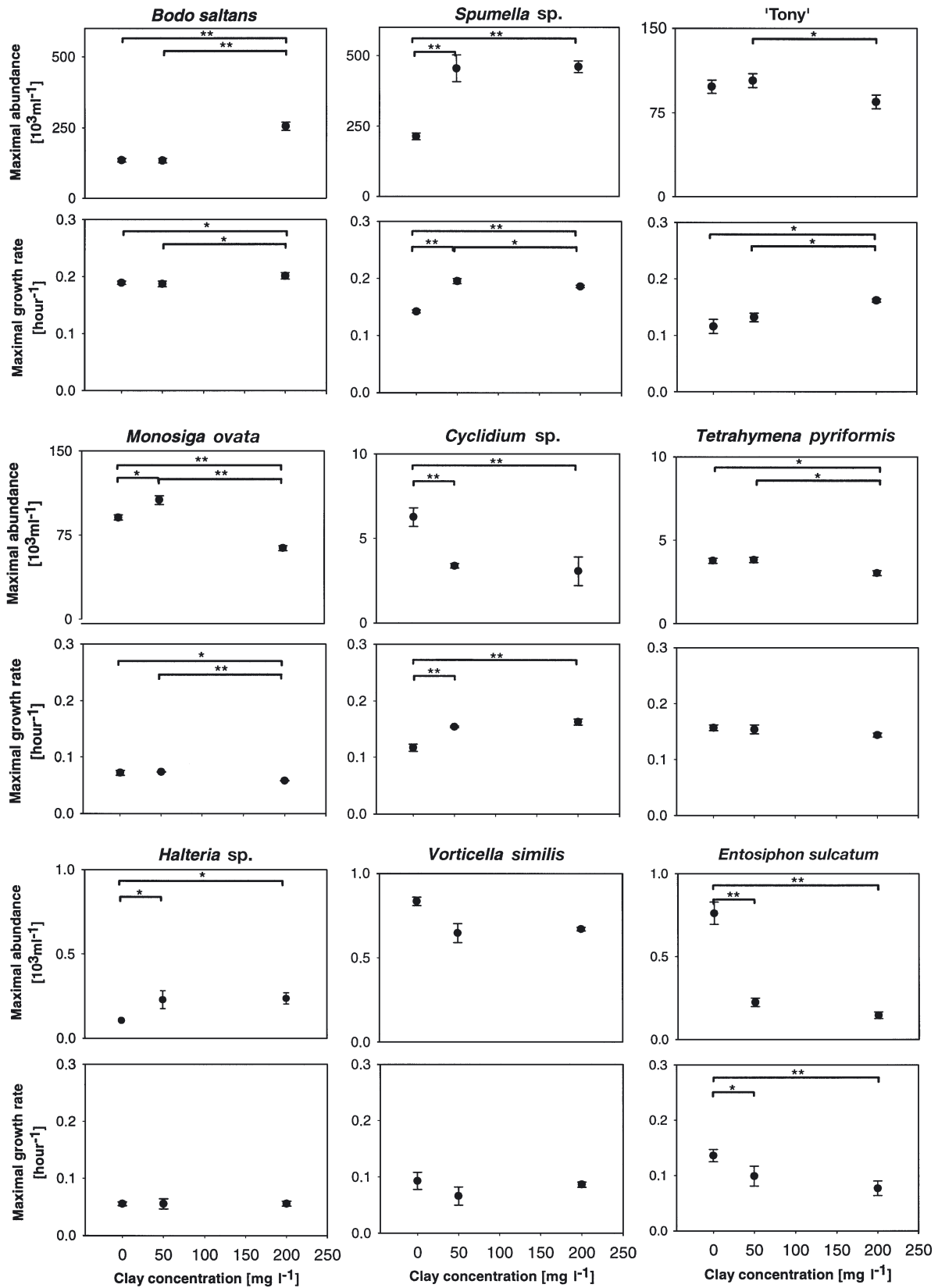


Fig. 2. Maximal abundance and maximal growth rates of bacterivorous protists in the presence of 0, 50, and 200 mg l⁻¹ suspended clay (mean \pm SD). Significant differences between the treatments: * α = 0.05; ** α = 0.001. Due to the high species-specific differences in the maximal abundance of the organisms scaling of the ordinate is different between species

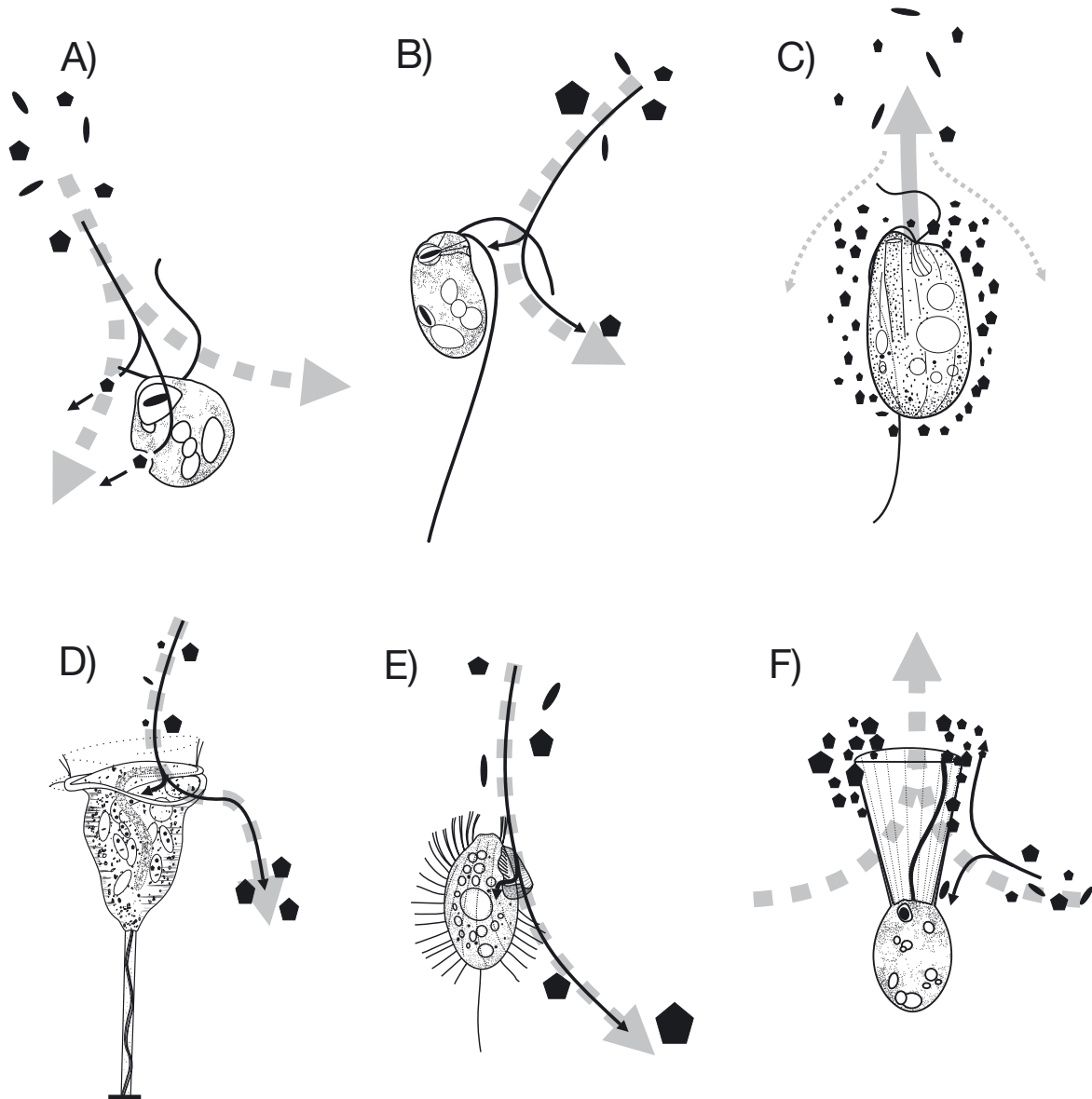


Fig. 3. Handling of clay (■) and bacteria (◄) by different feeding types of bacterivorous protists. Slashed grey arrows: water current; black lines: flow of particles; grey arrow: movement of the organism for *Entosiphon sulcatum* (A) *Spumella* sp.: selection prior to food uptake, as well as differential digestion occur; its relative importance depends on suspended sediment concentration (see 'Growth experiments'). (B) *Bodo saltans*: selection prior to food uptake is more effective, whereas differential digestion is generally of less importance. (C) *Entosiphon sulcatum*: a layer of clay particles covers the organism after a short period, consequently blocking contact with new food particles. (D) *Vorticella similis*: larger clay particles are not directed into food vacuoles even though *V. similis* does not seem to actively select. (E) *Cyclidium* sp.: particles larger than approx. $0.8 \mu\text{m}$ were individually handled. (F) *Monosiga ovata*: filtering is unselective but particles are then individually directed towards the cell body or the outer region of the collar

Bodo saltans

Strong but incomplete discrimination of clay particles in advance to food uptake was observed irrespective of the food concentration. Clay particles coming into contact with the flagellate were captured, but clay particles were usually rejected before ingestion. Some clay particles were, however,

ingested. Ingested clay particles did not visibly differ in form or size from those rejected by the flagellate. Food vacuoles often fused into larger vacuoles containing several food particles. The jerking movements of *Bodo saltans* did not allow for continuous observation of food vacuoles. Therefore differential digestion could not be observed regularly even though it occurred from time to time.

'Tony'

This protist occurred in 2 distinct morphological types, i.e. a biflagellated cell which was usually rotating at about 150 rotations min^{-1} , and an amoeboid form which was seen to creep on the substrate. Both types occasionally ingested clay particles even though most of the particles were not ingested. In the amoeboid form clay particles were ingested regularly, but excreted shortly afterwards. In the rotating flagellated form, food uptake and food vacuoles were difficult to observe owing to the cell's motion.

Entosiphon sulcatum

Entosiphon sulcatum usually glided attached to surfaces and searched for bacteria or detrital material. This behaviour was similar in the presence or absence of clay. This species seems to discriminate strongly in advance of food uptake. However, when clay was present many clay particles were seen to be loosely attached to the flagellate. The suspended material trailed by the flagellate when gliding decreased the food particle (bacteria) contact probability and ingestion rate (Fig. 3).

Monosiga ovata

Monosiga ovata discriminated strongly against clay particles prior to food uptake. Even though all kinds of particles were filtered and collected on the collar, particles were individually transported along the collar. Most clay particles were transported along the collar to the outer region where they were seen to accumulate. Even at high clay concentrations the collar was not covered completely with clay particles. About one-half of the filter structure remained particle-free, allowing for further feeding and selection. Clay particles were ingested occasionally. Even though *M. ovata* is morphologically a filter feeder, particles were individually handled and selected as it is typically so for interception feeders.

Cyclidium sp.

Cyclidium sp. ingested high amounts of clay and food vacuoles consequently contained a mixture of clay particles and bacteria. However, at least in the case of larger sized clay particles (>approx. 0.8 μm diameter), selection prior to food uptake was observed. These larger clay particles were observed to be handled individually and after successful capture they

were rejected by the ciliates without being incorporated into a food vacuole. For smaller particles individual rejection of particles could not be observed even though some discrimination between clay and bacteria might also have occurred. Mainly due to the individual rejection of larger clay particles, the amount of clay ingested was lower as compared to an unselective food uptake. As a result feeding and consequently food selection seemed to be intermediate between non-selective functional filter-feeding and selective functional interception-feeding.

Vorticella similis, *Tetrahymena pyriformis* and *Halteria* sp.

These species all ingested high amounts of clay particles, in contrast to the flagellate species and *Cyclidium* sp. Active particle selection could not be observed. However, larger clay particles in the filtration current were not captured successfully, nor directed into a food vacuole (Fig. 3). This may be due to hydrodynamic particle properties rather than active selection. Particles in the size spectrum of food particles were usually ingested, leading to a mixture of bacteria and clay inside each food vacuole. Consequently, differential digestion could not be observed. About 30 to 60 min after ingestion the remaining food vacuole content was excreted. Food vacuoles generally had a shorter 'lifetime' when high amounts of clay were ingested. During digestion the pellets were compacted, so they usually did not break up during excretion. Only in *Vorticella similis* did the excreted pellets break down when passing the cilia after excretion. The organisms contributed to a build-up of particle aggregates and reduced the abundance of single clay particles.

DISCUSSION

Suspended clay particles (<2 μm) are similar in size to bacteria. Therefore, it might be expected that they are able to interfere with the feeding behaviour of bacterivorous protists and cause a reduction of their growth rates. At the inception of this study a significant negative effect of suspended fine sediments on bacterivorous protists was expected. However, most species were not or only slightly affected and, surprisingly, several species actually showed increased growth rates and maximal abundances. This is a novel and unexpected finding.

In general, due to the masking of bacteria by clay particles bacterial abundance could only be followed during the growth experiment as long as it was above 10^7 bacteria ml^{-1} and no data on bacterial community

composition were collected. Some of the discussed effects may therefore be only indirectly linked to the suspended sediment concentration. Shifts in the bacterial community related to the addition of suspended sediment may be partly responsible for our findings. This is less likely for the maximal growth rate, as it was always calculated from the initial phase of the experiment when food concentration was assumed to be satiating. In contrast, the maximal abundance of the protists may have been indirectly affected by shifts in the bacterial community. Such indirect effects as well as a changed availability of organic substrates when attached to clays may be responsible for the surprising increased growth rates and maximal abundances in some species when suspended clay was present.

Organisms which handle food particles individually

These organisms are able to use a variety of mechanisms to select specifically between food and non-food objects, including differential particle handling prior to ingestion (Boenigk et al. 2002) and differential digestion (Boenigk et al. 2001). Therefore, it is expected that non-food objects will be discriminated against. For instance, in *Spumella* sp. a rapid excretion of ingested clay particles was observed (Zwart & Darbyshire 1992). Depending on the food concentration, differential digestion or differential particle handling become the predominant selection mechanism (Boenigk et al. 2002). However, a minimum effort is necessary to handle the particles, and therefore a slightly negative effect due to suspended clays was expected. Our results suggest that growth may be stimulated by the presence of clay. It is unclear whether this is a consequence of the direct use of organic substrates which may be attached to clay particles (Grant et al. 1997), or indirectly coupled to a stimulation of bacterial growth and thus a higher food concentration. However, the direct negative effect of wasting handling time and energy with handling of non-food particles was compensated in *Spumella* sp., *Bodo saltans*, and 'Tony', which were hardly affected by suspended clays, and showed even higher growth rates. The positive response of 'Tony' in terms of growth rate, but a negative response in terms of maximal abundance might be due to a changing energy budget for the feeding process. Suspended clay seems to increase metabolic costs thus decreasing the gross growth efficiency and consequently the maximal abundance. In contrast, the uptake of energy per time may be significantly higher thus allowing for higher growth rates and overcompensating the higher metabolic costs, as long as food is available. The selection mechanisms present in these species appear sufficient to deal with situations of high

particle load. One exception is the gliding flagellate *Entosiphon sulcatum*, which was seen with a covering of clay particles which could not be easily removed by the flagellate. This could be due to the steady-going, slow movements of this flagellate. However, this 'clay cover' hindered the (food) particles from coming in contact with the flagellate. Therefore the ingestion rate dropped and, consequently, the growth rate as well. This result was quite astonishing for the substrate-bound, gliding species, which might be expected to be well adapted to environments enriched with non-food particles. For these organisms it seems to be important whether sediment particles are freely suspended or aggregated.

Organisms which concentrate food particles

Captured particles are not handled individually by *Halteria* sp., *Vorticella similis* and *Tetrahymena pyriformis* and consequently strong food selection between food and non-food objects was not expected for these species, except for passive mechanisms (hydrodynamic forces related to particle size and particle density discrimination). Ciliates feeding on large prey items (e.g. algae) are able to actively reject unsuitable prey during the feeding process (Hausmann 2002). However, in ciliates feeding on smaller food items, and thus collecting food prior to ingestion, active food selection mechanisms are believed to play a minor role. For instance, it has been reported that inert surrogate food particles (beads) are not discriminated by the bacterivorous ciliate *V. similis* (Sanders et al. 1989). The comparison of a bacterivorous ciliate, i.e. *Uronema* sp., with an algivorous ciliate, i.e. *Strombidium sulcatum*, by Christaki et al. (1998) revealed a similar pattern. Whereas no selection between plain and carboxylate microspheres was noted for the bacterivorous ciliate in that study the algivorous ciliate selected for plain microspheres. Even though some abilities for selecting for prey characteristics other than size also seem to be present in bacterivorous ciliates (Christaki et al. 1998, 1999), food selection seems to be less pronounced compared to organisms with a lower predator-to-prey size ratio, i.e. algivorous ciliates and interception-feeding flagellates. The non-selective uptake of particles by bacterivorous ciliates, irrespective of their nutritional value should cause a marked decrease in the nutritional value, of ingested matter. Consequently, a drastic reduction of growth rates was expected. However, this reduction was only slight and not significant in all species. Owing to the fact that these organisms ingested particles irrespective of their nutritional value, clay represented the main component of the food vacuole content. Therefore, a much

stronger decrease of the growth rate with increasing clay concentrations was expected. This discrepancy suggests a high maximum capacity of food vacuoles, far exceeding the capacity usually required for food particles. In turn, high feeding capacity combined with an omnivorous feeding strategy on chiefly small particles, as it is known for small oligotrichs, might be a primary cause for the success of these organisms in pelagic waters. These have repeatedly been found to be the major bacterivores in lakes (Jürgens & Šimek 2000, Šimek et al. 2000). Several bacterivorous ciliates have been suggested to be adapted to high food concentrations (Fenchel 1980). At food concentrations which are usually found in natural environments, however, a high feeding capacity could compensate for non-selective ingestion of non-food objects and thus enable ciliates to compete with other bacterivores.

Intermediate feeding types (e.g. morphological filter feeders but functional interception feeders)

Monosiga ovata effectively handles particles more or less individually even though the organism possesses a filtering structure. Particles are held back by the filter unselectively, but are transported along the filtration structure individually. This individual particle transport was even observed for particles at and below the resolution level of the microscopical set-up (particles <0.2 µm in diameter; such particles were visible, but size measurements were not possible due to the resolution limits of light microscopy). Clay particles were seen to be transported to the outer region of the filter and accumulated there. By contrast, bacteria were often transported towards the cell body. Food particles exceeding approximately 0.1 µm³ were ingested individually, whereas smaller particles were concentrated prior to ingestion and then ingested into 1 food vacuole. Clay particles were ingested only occasionally. A similar behavioural flexibility, i.e. filter feeding on small particles but functional interception feeding on large ones, was observed for *Cyclidium* sp. (Pfandl et al. unpubl. data). For this reason the choanoflagellate *M. ovata* and the scuticociliate *Cyclidium* sp. were expected to be less affected by clay than other bacterivorous ciliates. However, clay was expected to clog the filter of *M. ovata* and to hinder other particles from coming within the proximity of the organism. Therefore, a negative overall effect was expected. However, the filter was not completely clogged even at high clay concentrations. Particle transport along the filter and disposal of clay particles was effective even at high concentrations of suspended sediment. Therefore, the growth of *M. ovata* was not strongly reduced in the presence of clay.

Implications for suspended sediment impacts on microbial communities in the field

The freshwater bacterivorous protists we investigated were generally well adapted to environments of high suspended sediment load. The idea that free-living protists are unable to distinguish between clay particles and bacteria (e.g. England et al. 1993) is not supported by our findings on the flagellates, whereas it is consistent with our results on ciliates. Active food rejection might be more important at high total particle load, i.e. when suspended fine sediment is present (Boenigk et al. 2002, this study), even though in some species it occurs even at low concentrations. This food selection mechanism could be mostly involved in distinguishing between food and non-food objects (Boenigk et al. 2002); its significance for selection between different bacterial strains still needs to be proved. Suspended sediments generally have been shown to affect aquatic organisms and to alter food web structure. Organisms which filter the water for food are usually more strongly affected than organisms which individually handle distinct prey items. Growth rate of cladocerans (Kirk & Gilbert 1990) and bivalves (Wilber & Clarke 2001), for example, decrease dramatically when high concentrations of suspended sediment particles which are the same size as potential prey are introduced. When suspended sediment concentrations exceed the threshold at which filter-feeding organisms can effectively filter material, the available food is diluted and the organisms may starve even at high food abundance (Widdows et al. 1979, Arruda et al. 1983).

From our results it appears that filter-feeding bacterivorous ciliates compensate for a low ability to select between clay and bacteria with a high feeding capacity. The heterotrophic microbial components of aquatic food webs appear to be generally less affected by suspended clay than the larger organisms (Jack et al. 1993). Some ciliate and flagellate species even responded positively to suspended sediment. In contrast to the generally negative effect of high suspended sediment loads, low concentrations of suspended sediments increase growth rates of filter feeders, as has been shown for bivalves at high food concentrations (Wilber & Clarke 2001) and for cladocerans at low food concentrations (Kirk & Gilbert 1990). It has been hypothesized that the increased growth rate is related to the use of nutritive organics, which are bound to the clay minerals as an additional food source (Arruda et al. 1983, Kirk & Gilbert 1990). Some protists may consequently better compete with bacteria for dissolved organic carbon when suspended sediments are present. Consequently, the energy input per unit time would be higher for protists feeding on a mixture of

bacteria and clay-attached organics. This could allow for a higher growth rate as seen in e.g. *Cyclidium* sp., 'Tony', and *Spumella* sp. A higher energy input per time, but also higher energetic costs due to particle handling, may be responsible for the findings of increased growth rate but decreased maximal abundance in 'Tony' and *Cyclidium* sp. It is, however, unknown if the protists and bacteria were able to exploit particle-attached organic substances.

Species-specific differences in the selection behaviour and in the relative importance of the different selection mechanisms (partly due to the feeding strategy, but occurring even within each particular feeding type) do occur in protists. Different protist species can therefore be expected to be differently affected by sediment input in surface waters. Similar to shifts in the metazoan community, e.g. from cladoceran-dominated communities to rotifer-dominated communities (Kirk & Gilbert 1990), changes in the protistan community are to be expected. The effects of the particle size and abundance as well as the influence of clay mineralogy and the role of particle-bound organics need, however, additional focus before predictions about the effects of suspended sediments on microbial populations can be made.

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