Effects of resuspension and mixing on population dynamics and trophic interactions in a model benthic microbial food web

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ABSTRACT: The effects of resuspension and mixing on the population dynamics and trophic interactions in a simple benthic microbial food web were studied during 3 plankton wheel experiments of 9 to 15 d duration. The food web consisted of a mixed bacterial assemblage, the heterotrophic flagellate *Bodo designis*, the ciliate *Euplotes balteatus* and the rhizopod *Vannella platypodia.* The diatom *Amphora coffeaeformis* was included to maintain oxygen concentrations. *B. designis*, *E. balteatus*, *V. platypodia* and *A. coffeaeformis* are hereafter referred to by genus name alone. Population dynamics in 500 ml microcosms on rotating and non-rotating plankton wheels were compared. Final abundances of *Amphora* increased in suspension in all experiments. Resuspension increased initial growth rates and final abundances of *Euplotes* and *Vannella* during a whole-community experiment and a community subset experiment during which resuspension effects with and without the top predator *Euplotes* were compared. During the community subset experiment, suspended *Bodo* grew faster and reached higher final abundances than non-resuspended *Bodo* when *Euplotes* was absent, but experienced higher loss to *Euplotes* when the ciliate was present. Individual consumption rates of *Bodo* by *Euplotes* were estimated to be higher in suspension (5.5 vs 3.6 *Bodo* $Euplotes^{-1}$ h^{-1}), which could not be explained by higher abundance of *Bodo* alone. Similar but non-significant trends were found for bacteria. During a third experiment, *Euplotes* did not benefit from resuspension at low food concentrations. Our results show that resuspension and mixing can enhance population growth of autotrophic and heterotrophic protists, and that the trophic coupling between flagellates and ciliates, and possibly between other microbial food web components, can become closer in suspension when the food supply is sufficient. This suggests that subsidiary energy input by resuspension and mixing may increase the biomass turnover in benthic microbial food webs.

KEY WORDS: Resuspension · Microbial food web · Trophic interactions · Growth enhancement · Ciliates · Rhizopods · Flagellates

INTRODUCTION

The sediment and water column of shallow lakes and coastal waters are closely coupled by sedimentation and wind- or tide-induced sediment resuspension. Together with resuspended sediments, bacteria, microphytobenthos and heterotrophic protists enter the water column during resuspension events (Shaffer & Sullivan 1988, Padišak et al 1990, De Jonge & Van Beusekom 1992, 1995, Arfi & Bouvy 1995, Shimeta & Sisson 1999, Garstecki et al. 2000). Although benthic protists can contribute substantially to water column abundances in the field (Shaffer & Sullivan 1988, De Jonge & Van Beusekom 1992, Garstecki et al. 2000), the effects of resuspension and mixing on resuspended protists and on the benthic microbial food web as a whole have not been studied. Instead, experimental studies of microbial responses to resuspension have shown important effects of released nutrients and organic carbon on plankton: phytoplankton can benefit from nutrients imported with resuspended sedi-

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ments (Kristensen et al. 1992, Reynolds 1996, Ogilvie & Mitchell 1998). The same is true for bacterioplankton (Wainright 1987, Jugnia et al. 1998) and heterotrophic nanoplankton (Wainright 1987).

The kinetic energy of wind and tides may favour plankton organisms through water column mixing and turbulence (e.g., Aguilera 1994, Peters & Gross 1994, Shimeta et al. 1995). Similarly, sediment resuspension and mixing should not only resuspend buried nutrients but also increase the total surface area of suspended particulates and alter encounter rates between predators and prey. Dilution of populations inhabiting resuspended sediments should decrease encounter rates, while mixing should increase them. This should result in increased bacterial growth and altered trophic coupling in resuspended benthic microbial food webs. As a first experimental test of this hypothesis, we have studied the effects of resuspension and mixing on a simple, artificially assembled benthic microbial food web. Although the experiments were designed to identify general mechanisms of resuspension on the microbial community, we have chosen benthic organisms that have previously been found in suspension in shallow inlets of the southern Baltic and in other shallow coastal waters (Zimmermann-Timm et al. 1998, Garstecki et al. 2000).

MATERIALS AND METHODS

Model community. The model benthic microbial community consisted of a natural mixed bacterial assemblage, the diatom *Amphora coffeaeformis* (length = 25 µm), the bacterivorous flagellate *Bodo designis* (length = 8 µm), the omnivorous ciliate *Euplotes balteatus* (length = 70 µm) and the presumably bacterivorous rhizopod *Vannella platypodia* (length = 15 µm, Expts 1 and 2 only). *B. designis*, *E. balteatus*, *V. platypodia* and *A. coffeaeformis* are hereafter referred to by genus name alone. Representatives of the genera *Bodo*, *Euplotes* and *Vannella* have been found in the water column of coastal waters during resuspension events (Garstecki et al. 2000, Zimmermann-Timm et al. 1998). The bacterial assemblage and all heterotrophic protists were isolated from sediments of Rassower Strom, coastal southern Baltic. Sediment aliquots were inoculated into Føyns-Erdschreiber medium (Page & Siemensma 1991). Protists were further isolated by dilution until all other eukaryotes were absent. Bacteria were separated from protists by filtration with a 0.8 µm polycarbonate membrane filter. The diatom *Amphora* was too large to be ingested by the other protists. It was included to maintain the oxygen concentration within the experimental bottles and to foster bacterial growth by exudation.

All stock cultures were kept at 18°C in Føyns-Erdschreiber medium, prepared with 10 PSU artificial seawater (ASW; Caron 1993). Illumination was 15 µmol m^{-2} s^{-1} PAR photon flux density (LI-COR quantum sensor, Biosciences, Lincoln, NB), with a light/dark cycle of 15/9 h.

Experimental design. The population dynamics of identical communities incubated together with autoclaved surface sediments on rotating and non-rotating plankton wheels were compared during 3 plankton-wheel experiments (Table 1). These consisted of a whole-community experiment with liquid organic enrichment, and 2 semi-continuous experiments, 1 with liquid organic enrichment and 3 different community subsets (community subset experiment), and 1 with identical communities but with or without particulate organic enrichment (particulate enrichment experiment). The experiments were carried out using vertical plankton wheels (1.2 m diameter). When rotating, the plankton wheels kept sediment and benthic organisms in suspension. The whole-community experiment was carried out to follow the overall dynamics of the microbial community in resuspended sediment. The microcosms were enriched once at the beginning of the experiment with Føyns-Erdschreiber medium (5%) to simulate nutrient and organic carbon pulses during resuspension events. During the community subset experiment we analysed how trophic interactions are affected by resuspension. The population dynamics of bacteria and nanofauna (*Bodo* and *Vannella*) with and without their respective predators (all heterotrophic protists in the case of bacteria and *Euplotes* in the case of *Bodo*) were followed, allowing estimates of loss rates due to predation in resuspended and non-resuspended communities. The particulate enrichment experiment was designed to distinguish resuspension effects mediated by an import of edible organic matter into the water column from resuspension effects without enrichment. *Urtica* powder was used to simulate particulate phytodetritus in this experiment.

Inoculation and incubation. Aliquots of the inocula were inoculated into 24 acid-rinsed 500 ml polycarbonate bottles containing 10 PSU ASW, 0.4 g l^{-1} double-sterilised, washed surface sediment from Rassower Strom and enrichment (Table 1). Before the whole-community experiment, an additional 4 flasks were prepared in the same way and sampled at t_0. The individual cultures for the community subset experiment were inoculated separately (Table 1). In this experiment, aliquots of 2 µm filtered cultures of *Vannella*, *Bodo* and *Euplotes* were pipetted into those bottles not receiving the respective inoculates. This was done to minimise differences in initial bacterial abundance due to bacteria co-inoculated with the protistan cul-

Table 1. Inocula, enrichments, initial abundances and sampling schedules. Each group in the community subset experiment and in the particulate enrichment experiment consisted of 4 resuspended and 4 control replicate bottles. Twelve control, 12 resuspended and 4 t_0 bottles were used in the whole-community experiment

	Whole community experiment	Community subset experiment			Particulate enrichment experiment	
		Group 1	**Group 2**	**Group 3**		
Inoculum	*Amphora*	*Amphora*	*Amphora*	*Amphora*	*Amphora*	
	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	
	Bodo		*Bodo*	*Bodo*	*Bodo*	
	Vannella		*Vannella*	*Vannella*		
	Euplotes			*Euplotes*	*Euplotes*	
					Group 1	**Group 2**
Enrichment	Soil extract (5%)	Soil extract (5%)			–	*Urtica* powder (50 mg l^{-1})
Initial abundance (ml^{-1})						
Amphora	270	3200	4600	2500	2500	2800
Bacteria	5.2×10^6	8.4×10^6	11.2×10^6	11.0×10^6	2.4×10^6	3.4×10^6
Bodo	660	–	3300	3000	2600	3000
Vannella	43	–	12	19	–	–
Euplotes	3.7	–	–	1.9	0.9	1.2
Sampling points (d)	0, 2, 7, 15	0, 2, 5, 9	0, 2, 5, 9	0, 2, 5, 9	0, 2, 5, 9	0, 2, 5, 9

tures. The initial abundances of bacteria, *Bodo*, *Vannella* and *Euplotes* were within the naturally occurring abundance range of the respective groups in the coastal southern Baltic (Garstecki et al. 2000). When closing the incubation bottles after inoculation, care was taken to prevent air bubbles from being trapped inside the bottles.

Eight or 12 bottles each were randomly mounted on 2 plankton wheels. In the whole-community experiment, all bottles received an identical inoculum. In the community subset experiment and in the particulate enrichment experiment, each wheel carried 2 or 3 groups of 4 replicate bottles, each group containing different community subsets or different enrichment levels. The plankton wheel carrying the resuspended microcosms rotated at 5 rpm, which was sufficient to keep the sediments in suspension. The wheel carrying the control microcosms only was turned once a day, by 180°, to correct for differences in light intensity on its upper and lower sides. The bottles mounted on the plankton wheels were incubated at 18°C and 15 µmol m^{-2} s^{-1} photon flux density (light/dark cycle 15/9 h) for 15 d (whole-community experiment) or 9 d (community subset experiment and particulate enrichment experiment).

Sampling and sample analysis. Before each sampling (Table 1), the bottles were extensively shaken and oxygen concentrations within the bottles were measured. Samples for the enumeration of bacteria, *Amphora*, *Bodo*, *Vannella* and *Euplotes* were taken. During the whole-community experiment, 4 replicate bottles were removed and sampled from each plankton wheel at each sampling occasion, whereas 100 ml of culture medium was sampled from each bottle at each occasion

during both semi-continuous experiments. Sample volumes were replaced with a cocktail of ASW, sediment and enrichment at concentrations equal to the initial concentrations in the respective bottles.

Oxygen concentrations were measured with an oxygen probe (WTW GmbH & Co. KG, Weilheim, Germany). Bacteria and *Bodo* were fixed with glutaraldehyde (1.5% final concentration) and enumerated in DAPI-stained samples using epifluorescence microscopy (Sherr et al. 1993). Briefly, 0.5 ml and 2 ml subsamples were stained (2.5 µg ml^{-1} final DAPI concentration) and concentrated on black polycarbonate filters (Millipore Corp., Bedford, MA) of 0.2 and 0.8 µm pore size for the enumeration of bacteria and *Bodo*, respectively. At least 50 *Bodo* and 300 bacteria on a minimum of 50 and 15 grids, respectively, were counted. In addition, the presence of picoautotrophs was checked based on chlorophyll autofluorescence at 450 to 490 nm excitation. *Vannella* was enumerated with a modified version of the liquid aliquot method (Garstecki & Arndt 2000). Between 24 and 72 subsamples of 1 to 100 µl were inoculated into wells of tissue culture plates containing Føyns-Erdschreiber medium. After 14 d incubation at 18°C in the dark, the wells were screened for the presence of *Vannella* and initial abundances were calculated from the frequency of positive wells assuming a Poisson distribution. *Euplotes* and *Amphora* were fixed in Bouin's fixative (a mixture of 80% formaldehyde saturated with picric acid and 20% acetic acid at 5% final concentration) and counted after sedimentation of 10 ml subsamples according to Utermöhl (1958). During the community subset experiment, aliquots of 1 ml from each ciliate-free microcosm were incubated in sterile

vials containing ASW enriched with soil extract and subsequently monitored for contamination with any heterotrophic protists (protist-free group) or *Euplotes* (*Euplotes*-free group).

Statistical analysis. Abundances were corrected for after-sampling replacement dilution in both semi-continuous experiments. Initial exponential growth rates were calculated from mean group abundances at t_0 and at 2 d, the first 2 samping points. When appropriate during the community subset experiment, loss rates due to predation were estimated from the difference in growth rate with and without predators present. Individual consumption rates of *Euplotes* were estimated from these loss rates, initial prey abundances and geometric mean predator abundances.

The significance of effects of time, resuspension, predators (community subset experiment only) and enrichment (particulate enrichment experiment only) was tested with log-transformed data using 2-way ANOVA (whole community experiment) or 2-way repeated measures ANOVA (RM-ANOVA; community subset and particulate enrichment experiment). The data were log-transformed for the tests because the variance differed between treatments, but the untransformed data are given in the figures. The significance of resuspension effects at individual sampling occasions was tested using unpaired *t*-tests, with Bonferroni corrections for the number of sampling occasions applied to the significance thresholds. Spearman's rank correlation coefficients between microbial abundances at all sampling occasions were calculated to gauge the degree of coupling within the microbial communities. All statistical procedures were carried out using SPSS (SPSS Inc., Chicago, IL).

RESULTS

Whole-community experiment

During the whole-community experiment, clear resuspension effects were found on several levels of the model microbial food web (Fig. 1). *Euplotes* showed a dramatic increase in abundance that was even greater in the resuspended bottles, but these effects were apparent only on the last sampling date (68.0 ± 5.1 and 28.1 ± 15.0 ind. ml^{-1}, resuspended and control microcosms, respectively, $p = 0.016$; Fig. 1e). *Vannella* also grew rapidly in both the resuspended and the control
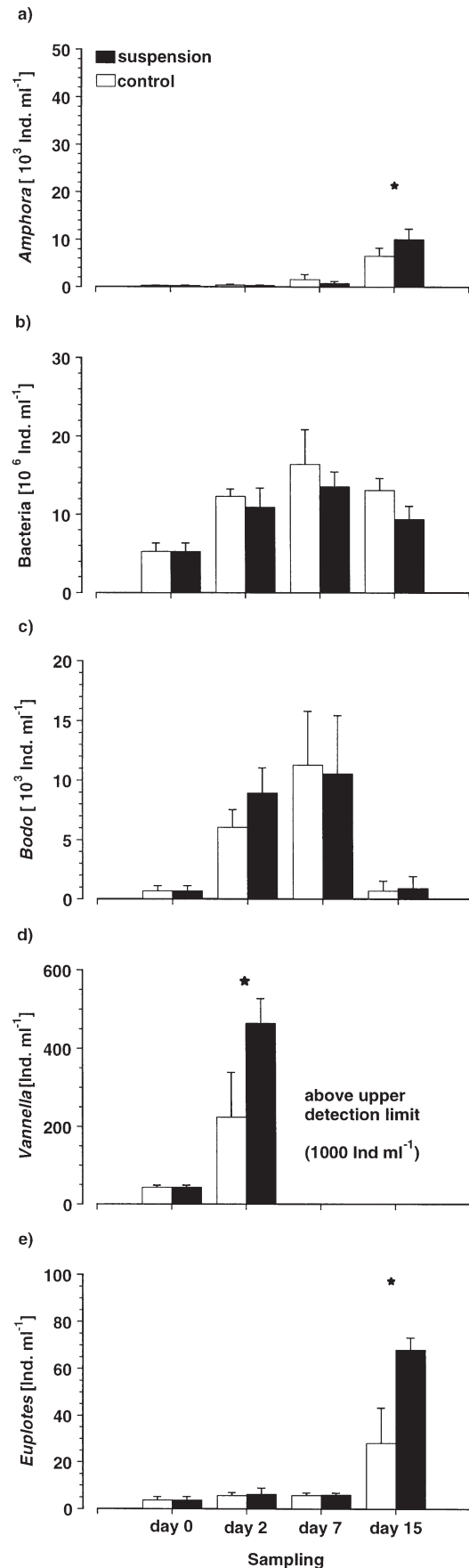

Fig. 1. Abundances of (a) *Amphora*, (b) bacteria, (c) *Bodo*, (d) *Vannella* and (e) *Euplotes* during the whole-community experiment (mean ± SD, N = 4). *p < 0.05, *t*-test, Bonferroni corrected

bottles (Table 2, Fig. 1d). They exceeded the upper detection limit of the cultivation method (1000 ind. ml^{-1}) after 5 d. However, their abundances were already significantly higher in the resuspended microcosms than in the controls after 2 d (465 ± 62 and 224 ± 115 ind. ml^{-1}, p = 0.016). Similar to *Euplotes*, *Amphora* showed an initial growth lag and was more abundant in the resuspended microcosms at the end of the experiment (10.1 ± 2.2 × 10^3 and 6.6 ± 1.7 × 10^3 ind. ml^{-1}, p = 0.025; Fig. 1a). Bacteria showed no significant resuspension effect (Fig. 1b). *Bodo* increased until Day 7 (maximum 11.3 × 10^3 ind. ml^{-1} in the control microcosms) and had declined by Day 15. Positive resuspension effects on initial growth rates (Table 2) did not result in significant differences in abundance at the end of the experiment (Fig. 1c).

Spearman's correlation coefficients suggest that population dynamics within the microbial community were related to each other in a complex manner: the abundances of *Amphora* and *Euplotes* were positively correlated over the whole course of the experiment (p < 0.01), while flagellate abundances were negatively correlated to those of the former groups (p < 0.01). Rhizopods were not included in this correlation analysis because they were counted only at the first 2 sampling occasions.

Community subset experiment

The general trends of the whole-community experiment were also found in the community subset experiment. Growth of *Amphora* lagged initially and was enhanced in suspension irrespective of microbial com-

Table 2. Mean initial growth rates of microbial populations in the community subset experiment (calculated from abundances on Day 0 and Day 2)

Treatment	Bacteria (d^{-1})	*Bodo* (d^{-1})	*Vannella* (d^{-1})	*Euplotes* (d^{-1})
Whole community				
Control	0.43	1.11	0.91	0.20
Suspension	0.37	1.30	1.28	0.30
Community subset				
Control	0.44	–	–	–
Suspension	0.40	–	–	–
Control + nanofauna	0.23	0.20	0.47	–
Suspension + nanofauna	0.27	0.27	0.92	–
Control + all protists	0.16	0.10	0.42	0.12
Suspension + all protists	0.16	0.06	0.53	0.39
Particulate enrichment				
Control	0.24	0.24	–	0.47
Suspension	0.15	0.25	–	0.03
Control + enrichment	0.27	0.46	–	0.25
Suspension + enrichment	0.27	0.53	–	0.43

Table 3. p-values for protistan and suspension effects over time and their interactions within the repeated measures (RM)-ANOVA design of the community subset experiment (N = 4). Prot.: protist presence; Susp.: suspension. *Presence of no protists, only *Vannella* and *Bodo*, or all protists in the case of *Amphora* and bacteria (2 × 3 RM-ANOVA), presence or absence of *Euplotes* in the case of *Bodo* and *Vannella* (2 × 2 RM-ANOVA). Values in **bold** show statistically significant results

	Amphora	Bacteria	*Bodo*	*Vannella*
Susp.	**0.011**	0.819	**0.024**	**<0.001**
Prot.*	0.811	**<0.001**	**<0.001**	0.367
Time	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Susp. × Prot.	0.429	0.333	**0.018**	0.658
Susp. × Time	**<0.001**	0.763	0.105	**0.002**
Prot. × Time	**0.015**	0.114	0.771	**0.034**
Susp. × Prot. × Time	0.125	0.627	0.976	**0.006**

munity structure (RM-ANOVA, Fig. 2, Table 3). In contrast, no significant protistan effects on diatom abundance were found. Final diatom abundances ranged from 6.7 ± 2.1 × 10^3 ind. ml^{-1} in the control microcosms with nanofauna only (Fig. 2b) to 33.1 ± 12.8 × 10^3 ind. ml^{-1} in the resuspended whole-community microcosms (Fig. 2c).

Similar to the whole-community experiment, *Euplotes* tended to grow faster in the resuspended microcosms (Fig. 3, Table 2). The final difference in abundance (75.7 ± 15.8 and 65.1 ± 19.8 ind. ml^{-1}, resuspended and control microcosms, respectively) was not significant in this experiment, however.

In the absence of ciliates, both *Bodo* and *Vannella* tended to grow faster in suspension (Fig. 4, Table 2). The final difference in abundance was significant for the flagellates (12.9 ± 2.6 × 10^3 and 6.9 ± 2.6 × 10^3 ind. ml^{-1}, resuspended and control microcosms, respectively, p = 0.024; Fig. 4a) but not for the rhizopods (284 ± 70 and 189 ± 83 ind. ml^{-1}; Fig. 4c). In the presence of *Euplotes*, net growth rates of *Bodo* were less and lower in suspension than in the controls (Table 2). There was no significant difference in final abundance of *Bodo* in resuspended and control microcosms in the presence of *Euplotes* (Fig. 4b). The RM-ANOVA of flagellate abundance revealed both significant main effects of ciliate presence and resuspension, and a significant interaction between both factors (Table 3). Estimated individual grazing rates of *Euplotes* on *Bodo* were high during the first 2 d of the experiment (3.6 and 5.5 *Bodo* *Euplotes*$^{-1}$ h^{-1}, control and resuspended microcosms, respectively) and declined to <0.1 *Bodo* *Euplotes*$^{-1}$ h^{-1} towards the end of the experiment when the average ratio of flagellate to ciliate abundance dropped below 10^3.
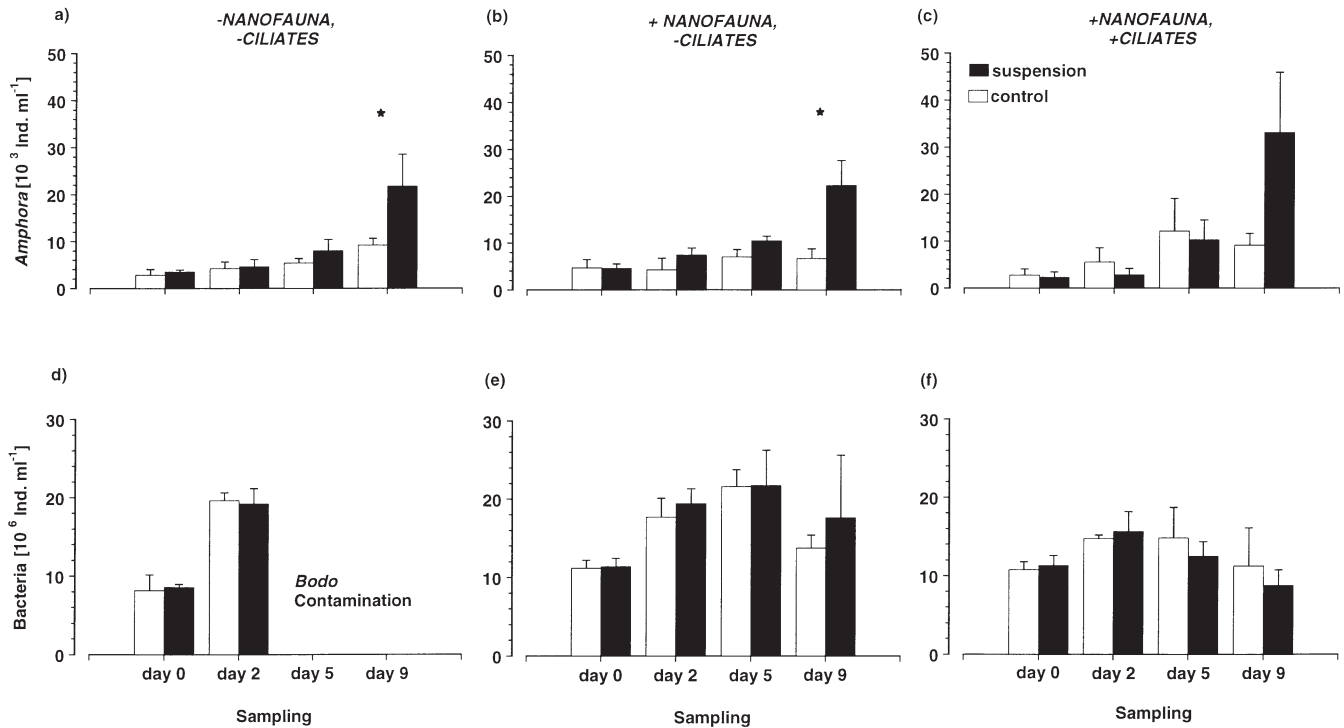
Fig. 2. Abundances of (a to c) *Amphora* and (d to f) bacteria during the community subset experiment (mean ± SD, N = 4). *p < 0.05, *t*-test, Bonferroni corrected. The scale for the *y*-axis is the same in the 3 experimental treatments

Vannella growth showed stronger positive resuspension effects in the presence of *Euplotes*, reaching 459 ± 113 ind. ml^{-1} at the end of the experiment, and significantly exceeding the abundances in the non-resuspended microcosms (182 ± 53 ind. ml^{-1}, p = 0.012; Fig. 4d). The RM-ANOVA of rhizopod abundances showed a highly significant positive resuspension effect, but no effect of ciliate presence and no interaction (Table 3).

Resuspension did not significantly affect bacterial abundance in any treatment combination (Fig. 2, Table 3). After 2 d, most of the protist-free microcosms were contaminated with *Bodo.* Therefore, the initially

Fig. 3. Abundance of *Euplotes* during the community subset experiment (mean ± SD, N = 4)

bacterivore-free group was excluded from further analysis. Treatments with ciliates had significantly fewer bacteria than those with only nanofauna or bacteria alone (Fig. 2f, Table 3). Although no grazing rates of *Euplotes* on bacteria could be calculated from our experiment, mean loss rates to bacterivores were much higher in the presence of all protists including *Euplotes* (−0.26 d^{-1}) than in the presence of *Bodo* and *Vannella* alone (−0.18 d^{-1}), suggesting that *Euplotes* had a strong grazing impact on bacteria.

In those microcosms where all eukaryotes were present, their abundances were significantly positively correlated to each other over the whole course of the experiment. Bacterial abundances were positively correlated to those of *Bodo* only (Table 4). Correlations between *Amphora*, *Vannella* and *Euplotes* were stronger in suspension, while correlations between bacteria on one hand and *Vannella* and *Euplotes* on the other were switched from positive to negative (data not shown). In microcosms without *Euplotes*, all microbial populations were significantly positively correlated to each other (Table 4).
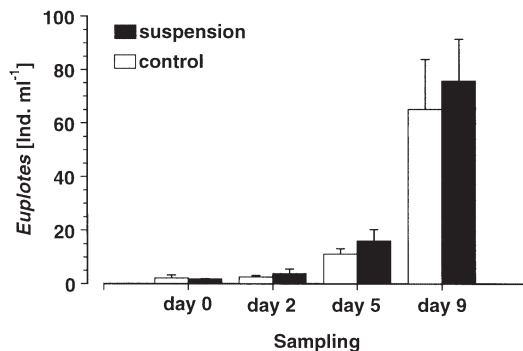
Particulate enrichment experiment

Urtica powder had a negative growth effect on *Amphora* (Fig. 5a,b, Table 5), but growth was again
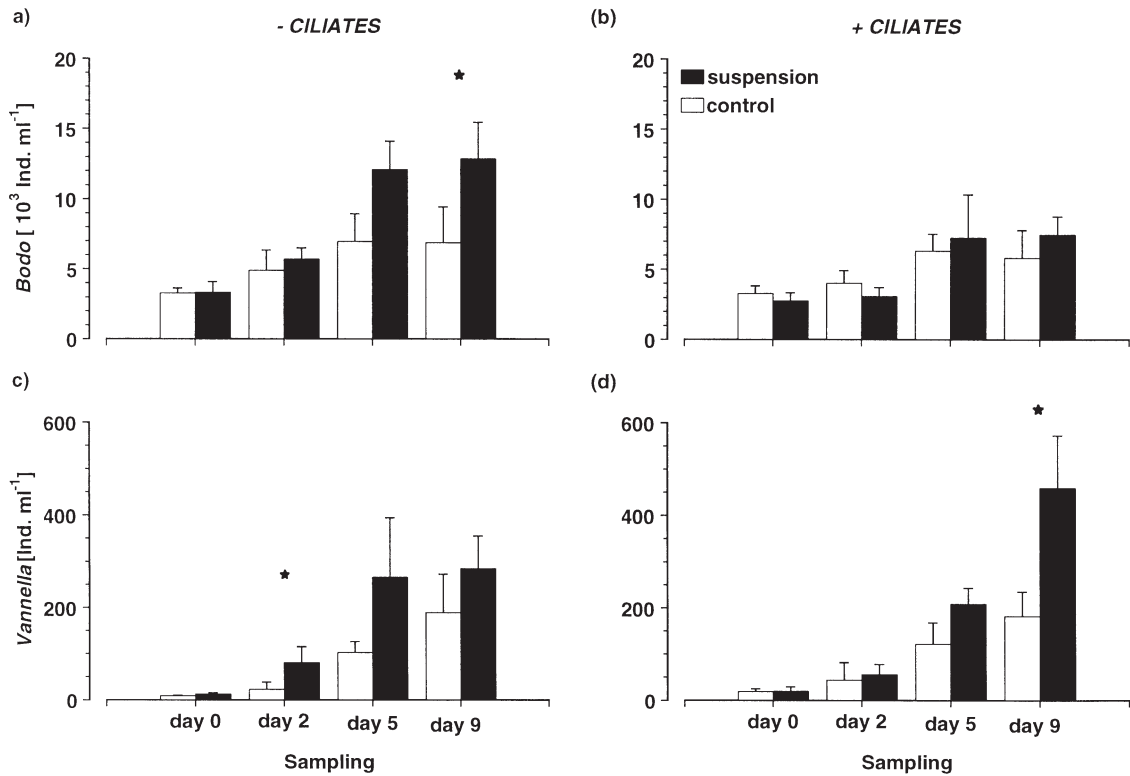
Fig. 4. Abundances of (a,b) *Bodo* and (c,d) *Vannella* in the presence (+ *Ciliates*) and in the absence (– *Ciliates*) of *Euplotes* during the community subset experiment (mean ± SD, N = 4). *p < 0.05, *t*-test, Bonferroni corrected. The scale for the *y*-axis is the same in both experimental treatments

fostered by resuspension in the non-enriched microcosms. Resuspension increased mean final diatom abundance in minimal medium by a factor of 3 compared with the final abundance in the non-resuspended controls ($37.9 \pm 5.8 \times 10^3$ and $11.6 \pm 2.9 \times 10^3$ ind. ml^{-1}, p = 0.004; Fig. 5a). Abundances of bacteria and *Bodo* were not affected by resuspension (Tables 2 & 5).

Compared with the minimal medium, particulate enrichment increased the final abundance of *Euplotes* (Fig. 5g,h, Table 5). Resuspension had a positive, time-dependent effect on ciliate growth and abundance, but only in the particle-enriched microcosms (final abundances of 25.5 ± 1.2 ind. ml^{-1} in suspension and 16.0 ± 3.3 ind. ml^{-1} in the controls, p = 0.012; Fig. 5h). It had a negative effect in minimal medium (final abundances of 6.9 ± 0.9 ind. ml^{-1} in suspension and 11.0 ± 1.9 ind. ml^{-1} in the controls, p = 0.028; Fig. 5g). This resulted in a significant interaction between particulate enrichment and resuspension in the RM-ANOVA (Table 5).

The abundances of *Amphora*, bacteria and *Euplotes* were positively correlated over the whole course of the particulate enrichment experiment (Spearman's r, p < 0.01), but neither enrichment nor resuspension had effects on these correlations. An exception to this trend

was the decoupling of bacteria and *Amphora* in the enriched microcosms by resuspension (Spearman's r without suspension 0.682, p < 0.004; in suspension 0.433, p = 0.094).

No effects of resuspension, community structure or enrichment on oxygen concentrations in the microcosms or on the size or shape of any organisms were found in any experiment. This was also true for the bacteria, which were dominated by rods of 1 to 2 μm length in all treatments. No picoautotrophs were present. Table 6 summarises resuspension effects on the final abundances of microbial food web components.

Table 4. Correlations between microbial abundances during the community subset experiment. Correlation coefficients for the whole community microcosms are given in the lower left-hand corner while those for the ciliate-free microcosms are given in the upper right-hand corner. Spearman's r; *p < 0.05; **p < 0.01)

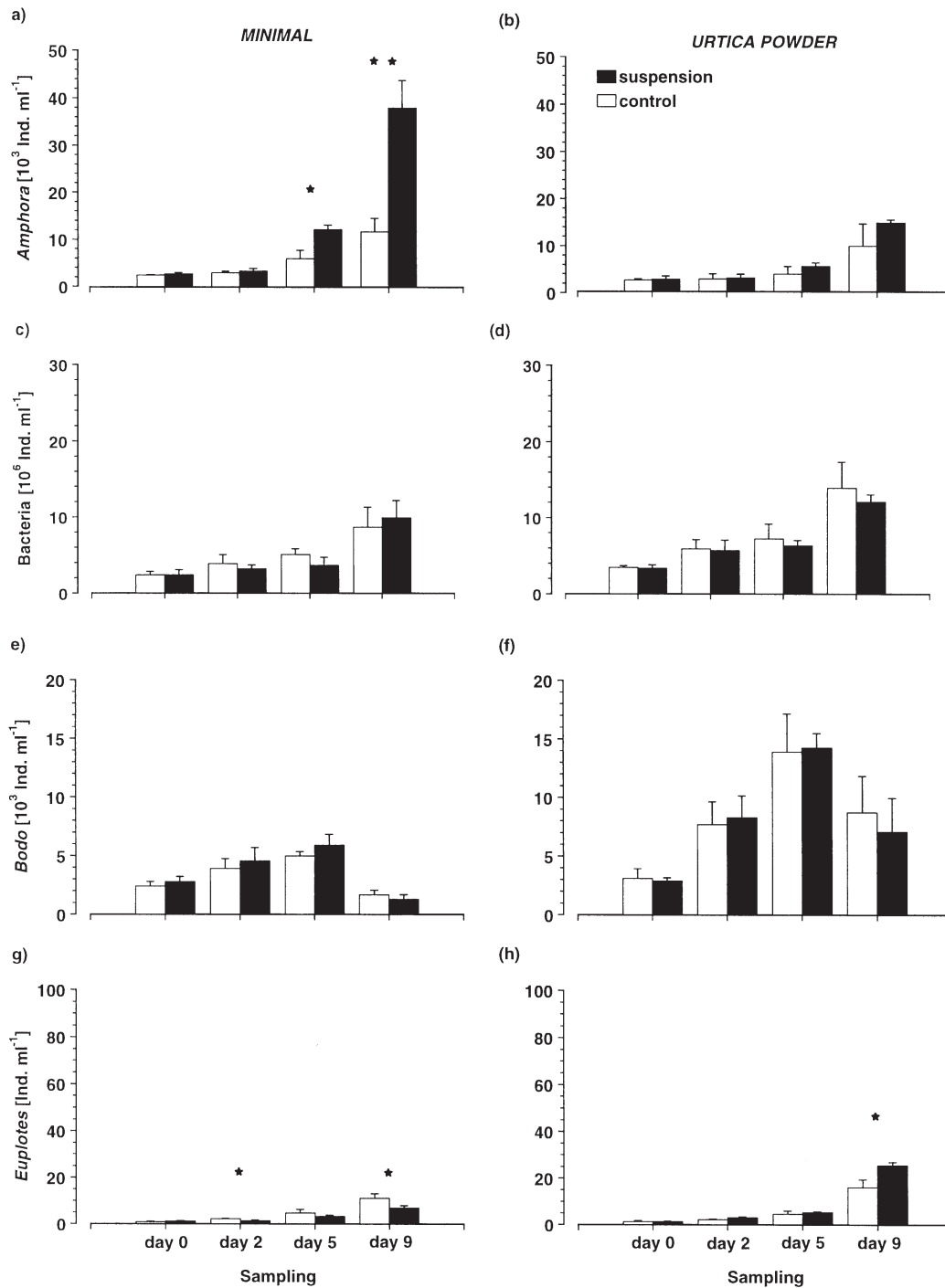|	*Amphora*	Bacteria	*Bodo*	*Vannella*
Amphora	–	0.396*	0.575**	0.763**
Bacteria	−0.077	–	0.915**	0.482**
Bodo	0.362*	0.793**	–	0.693**
Vannella	0.704**	−0.173	0.405*	–
Euplotes	0.783**	−0.103	0.478**	0.893**

Fig. 5. Abundances of (a,b) *Amphora*, (c,d) bacteria, (e,f) *Bodo*, and (g,h) *Euplotes* with (*Urtica powder*) and without (*Minimal*) enrichment during the particulate enrichment experiment (mean ± SD, N = 4. *p < 0.05, *t*-test, Bonferroni corrected. The scale for the *y*-axis is the same in both experimental treatments

DISCUSSION

Food web structure

The results of our experiments, especially those of the community subset experiment, support the food web structure that was derived from direct observation and literature data (e.g., Pratt & Cairns 1985, Premke & Arndt 2000). The abundances of bacteria and *Bodo designis*, but not those of *Amphora coffeaeformis* and *Vannella platypodia*, decreased in the presence of *Euplotes balteatus*. This is consistent with bacterivory

Table 5. p-values for resuspension and enrichment effects over time and their interactions within the RM-ANOVA design of the particulate enrichment experiment (N = 4), Susp.: suspension; Enr.: enrichment. Values in **bold** show statistically significant results

	Amphora	Bacteria	*Bodo*	*Euplotes*
Susp.	**0.003**	0.113	0.465	0.481
Enr.	**0.014**	**<0.001**	0.440	**<0.001**
Time	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Susp. × Enr.	0.277	0.777	0.580	**<0.001**
Susp. × Time	**<0.001**	0.330	0.593	0.693
Enr. × Time	**0.001**	0.667	0.787	**<0.001**
Susp. × Enr. × Time	0.077	0.305	0.819	**<0.001**

Table 6. Summary of resuspension effects on final microbial abundances. No significant resuspension effects on bacteria were found. +: positive resuspension effect; –: negative effect; 0: no significant effect; a: absent. *After 2 d

Group	*Amphora*	*Bodo*	*Vannella*	*Euplotes*
Whole-community experiment				
	+	0	+*	+
Community subset experiment				
–*Euplotes*	+	+	0	a
+*Euplotes*	0	0	+	0
Particulate enrichment experiment				
–Enrichment	+	0	a	–
+Enrichment	0	0	a	+

and predation on *Bodo* by *Euplotes*, trophic links that are well known from previous studies. Consumption rates of up to 16×10^3 bacteria ind.$^{-1}$ h^{-1} (*Euplotes mutabilis*) and up to 21 *Bodo sorokini* ind.$^{-1}$ h^{-1} (*Euplotes vannus*) from prey concentrations similar to those used in our experiments have been reported (Zubkov & Sleigh 1996, Premke & Arndt 2000).

Bodo designis and *Vannella platypodia* are also bacterivores (Pratt & Cairns 1985, Page & Siemensma 1991) and were expected to reduce bacterial abundances in the absence of *Euplotes*. However, we did not find evidence for this interaction. A possible explanation is that *Vannella* fed on *Bodo*, in addition to bacteria. Thus, *Vannella* would have contributed less to bacterivory, while keeping abundances of bacterivorous *Bodo* relatively low. Predation on heterotrophic protists by *Vannella* has not been studied, but it has been reported from other rhizopods (e.g., Bovee 1960), and predation on *Bodo* by *Vannella* has occasionally been observed in crude cultures (Garstecki pers. obs.). Although *Vannella* does not appear to be an efficient flagellate grazer, the interaction may explain the crash in *Bodo* abundance at the end of the whole-community experiment when abundance of *Vannella* was exceptionally high.

In addition to trophic interactions between microbial populations, the links between experimental enrichment and the microbial food web need to be considered. *Amphora coffeaeformis* was not favoured by enrichment. Particulate enrichment with *Urtica* powder, a particulate phytodetritus surrogate, enhanced population growth of all heterotrophic protists (Table 5), but it is not clear at which trophic level it was incorporated into the microbial food web. Bacterial utilisation after enzymatic breakdown, direct protistan detritivory (Posch & Arndt 1996) and a combination of both are possible. The temporal resolution of our data set is not sufficient to distinguish between these trophic links or between mechanisms of liquid enrichment utilisation. Nevertheless, our data confirm the predicted general food web structure. Against the background of this structure, effects of resuspension on individual trophic links can be discussed.

Resuspension effects on *Amphora coffeaeformis* and bacteria

De Jonge & van Beusekom (1995) assumed that suspended microphytobenthos grow as efficiently as sedimented ones. However, resuspension increased the abundance of *Amphora* during all of our experiments. Since enrichment was identical in resuspended and control microcosms in the first 2 experiments, and since resuspension tripled final diatom abundances in microcosms without enrichment, it is unlikely that there were insufficient nutrients to support diatom growth in the static controls. Rather, the ability of *Amphora* to utilise the nutrients present in the medium was likely higher when they were in suspension. The more efficient nutrient utilisation could be due to increased mean nutrient supply to individual cells in a population dispersed by resuspension (Bothwell 1989) or to reduced transport limitation of nutrient supply to suspended cells. While similar effects of water motion on phytolankton nutrient uptake and growth are well established (Aguilera et al. 1994, Reynolds 1994, Estrada & Berdalet 1997), they have not yet been discussed with respect to resuspension effects on microphytobenthos.

Increased light attenuation after resuspension may decrease microphytobenthos primary production in natural systems (Hellström 1991, MacIntyre & Cullen 1996). However, the net resuspension effect on water column primary production is also influenced by decreased light attenutation due to reduced self-shading, and nutrient and chlorophyll *a* fluxes from the sediment, and is thus not necessarily negative (Gabrielson & Lukatelich 1985, MacIntyre & Cullen 1996). Light attenuation by suspended particulates

was generally taken into account in our experimental design because sediment was included in the microcosms. Nevertheless, growth of *Amphora* appeared not to be reduced due to insufficient light in suspension. It remains to be seen whether enhanced growth due to better nutrient access in suspension is a general phenomenon of resuspended benthic algae, and how different light regimens affect the response of these algae and further effects on the microbial food web.

The failure to detect a positive resuspension effect on bacterial gross growth (Fig. 2d) and abundance during our experiments may partly be due to the limited accuracy of our methodology. However, planktonic bacteria did not show direct effects of experimental turbulence and mixing in previous studies, and solitary bacterial cells are considered too small to benefit directly from water motion (Moeseneder & Herndl 1995, Karp-Boss et al. 1996, Peters et al. 1998). Therefore, it is unlikely that enhanced bacterial utlisation of dissolved organic matter and increased bacterial gross growth rates in suspension contributed to the positive resuspension effects that were observed at higher trophic levels in our experimental food web.

Resuspension effects on protistan population dynamics

Resuspension enhanced the population growth of *Euplotes balteatus* in all enriched experiments except the minimal ASW medium, where there was a significant decline. This pattern is consistent with improved food access or increased growth efficiency of *Euplotes* in suspension when food is generally abundant and impaired food access or reduced growth efficiency when food is scarce. The results of the community subset experiment support the food accessibility hypothesis: the increase in the abundance of *Bodo designis* in resuspended versus control treatments disappeared when *Euplotes* was present. A similar, but non-significant trend was found for *Euplotes* interactions with bacterial abundance: in the community subset experiment, there was lower abundance in resuspension only when ciliates were present. In the other experiments where ciliates were present in all treatments, there was again the pattern of higher ciliate but lower bacterial abundance in resuspended microcosms. Only in the unenriched treatments of the particulate enrichment experiment did this pattern not hold. The higher grazing impact of *Euplotes* in suspension was not due to increased prey abundance and higher functional responses alone because numerical predator/prey ratios were always higher in suspension, and bacterial abundances became lower in suspension than in the controls after 5 d. Suspended, dispersed prey may be

more accessible for an average individual in a filter-feeding *Euplotes* population than discrete, surface-associated food patches, and mixing may increase prey encouter rates (Shimeta et al. 1995, Peters et al. 1998).

The data of the particulate enrichment experiment agree with a trophic mechanism mediating resuspension effects on population dynamics of *Euplotes*. The negative resuspension effect on ciliate growth in the minimal ASW group shows that this mechanism operates only above a certain food concentration and may be non-existent at low food supply in the field. Since abundances of bacteria and *Bodo* were low without enrichment, resuspension and dispersal may have decreased prey encounter rates in this group until grazing in suspension was less efficient than grazing from patches of sedimented prey in the non-resuspended treatment. Zubkov & Sleigh (1996) reported a drop of gross growth efficiency from 43 % to 20 % in *Euplotes mutabilis* as bacterial food concentration was decreased from 30×10^6 ml^{-1} to 6×10^6 ml^{-1}. The relevance of food supply for ciliate growth during the particulate enrichment experiment is highlighted by positive correlations of bacterial and ciliate abundances that are consistent with bottom-up control of *Euplotes balteatus* by bacterial food supply.

The effects of resuspension on trophic links involving *Euplotes balteatus* cannot be too widely generalised because they depend partly on the feeding ecology of this ciliate. However, another hypotrich ciliate (*Aspidisca* sp.) also responded positively to sediment disturbance in earlier experiments (Alongi 1985). The rhizopod *Vannella platypodia* and the flagellate *Bodo designis* responded positively to resuspension during our experiments although they have a surface-associated feeding mode. They were probably associated with aggregates in suspension, as has been observed both in the field and in the laboratory (Artolozaga et al. 1997, Zimmermann-Timm et al. 1998). Trophic mechanisms of resuspension effects involving *Vannella* and *Bodo* are difficult to discern because they were always inoculated together, or together with *Euplotes* in the case of *Bodo* in the particulate enrichment experiment. The rhizopods were positively correlated to bacteria and *Bodo* in the community subset experiment when *Euplotes* was absent. This may imply bottom-up control of *Vannella* by bacterial and possibly flagellate food supply, and a similar mechanism to that in *Euplotes* leading to improved food access in suspension. Gross growth rates of *Bodo* were much higher in suspension, but most of the surplus biomass in resuspended microcosms was consumed by *Euplotes* when the ciliate was present. Thus, *Bodo* had a higher biomass turnover in suspension and was a more efficient link in terms of carbon flow. Since *Euplotes* and *Vannella* were the top predators in our food web and

Amphora was not consumed, biomass accumulated in their populations towards the end of the experiments. If higher trophic levels had been present in the microcosms, biomass turnover of *Amphora*, *Euplotes*, and *Vannella* might have increased in suspension similar to that of *Bodo*. This would have resulted in enhanced carbon flow through the experimental food web.

Our experimental design was focused on the effects of resuspension and mixing effects on trophic interactions and population dynamics in a model microbial food web. Different benthic species may react differently to resuspension, but the organisms used are major components of the microbial food web in coastal Baltic sediments (Garstecki et al. 2000) and have all been found associated with particulates in the water column (Zimmermann-Timm et al. 1998). Sediment concentrations, initial abundances and light availability were set at levels close to field conditions. Although the experimental enrichment levels may have been higher than the organic input to the water column during natural resuspension events, an increase of organic carbon and nutrient concentrations is one of their consequences: substantial stimulations of bacterial population growth by dissolved and particulate carbon inputs from the benthos have been found in various field and laboratory studies (Wainright 1987, Ritzrau & Graf 1992, Hopkinson et al. 1998). Wainright (1987) also reported increased protozoan community biovolume after addition of resuspended material, and Ogilvie & Mitchell (1998) detected nutrient-mediated positive effects of sediment resuspension on phytoplankton.

In contrast to the focus on transport effects in the abovementioned studies, our experiment focused on the impact of mixing and suspension at a given nutrient and carbon input. The impact of these processes on resuspended benthic microbial food webs has not yet been studied, but effects of mixing and turbulence on planktonic microbial food web interactions have been addressed (Peters & Gross 1994, Shimeta et al. 1995, Peters et al. 1996, 1998). Although laminar shear was not quantified in our experiments, the designs of our experiments and the plankton studies both included mixing and dispersal, and can be compared with respect to their effects. Shimeta et al. (1995) found increased clearance rates in cultures of the choanoflagellate *Monosiga* sp. and the heliozoon *Ciliophrys marina* but decreased clearance rates in the tintinnid ciliate *Helicostomella* sp. when they were subjected to laminar shear below the turbulence microscale. They hypothesised that non-motile protists and weak swimmers would benefit most from increased turbulence and mixing, which is in agreement with the pronounced growth enhancement of the rhizopod *Vannella* during our experiments. Peters et al. (1998)

reported bacterial net growth enhancement under turbulent conditions due to shifts of food size spectra of planktonic grazers towards larger prey. Consistent with this result, estimated consumption rates of *Bodo designis* by *Euplotes balteatus* were higher in suspension during our community subset experiment, but the negative effect of protistan grazers on bacterial abundance was still stronger in suspension. Although turbulence did not increase individual grazing rates of the heterotrophic flagellate *Paraphysomonas imperforata* during laboratory experiments, enhanced growth rate, decreased mean cell size and possibly behavioural adaptations resulted in a higher bacterial consumption of the flagellate population under turbulent conditions (Peters et al. 1996). Similar net effects were found in a natural nanoplankton assemblage (Peters & Gross 1994).

Our results show that mixing during resuspension events may also have profound effects on the dynamics of resuspended benthic microbial food webs. The subsidiary energy input by resuspension and mixing may increase gross growth rates and consumption rates by higher trophic levels. This may result in a higher carbon turnover of benthic microbial food webs in shallow ecosystems.

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