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The role of nutrients in decomposition of a thecate dinoflagellate

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Abstract

The decomposition of freeze-dried whole cells and empty thecae of the dinoflagellate *Peridinium gatunense* Nygaard originating from dense blooms in Lake Kinneret (Israel) was followed experimentally under controlled conditions in the lab. The two materials (whole cells; empty thecae) were suspended in replicate bottles containing nutrient-poor epilimnetic water from the lake. After 7 d, nutrients (N, P, and trace metals) were added to half the bottles. We followed the changes with time in dry weight, the dynamics of nutrients, microbial abundances and a range of microbial activities including leucine incorporation rates and activities of hydrolytic enzymes. Because of the low N and P content of thecae (C : N : P atomic ratios >3000 : 19 : 1) relative to protoplasts (276 : 51 : 1), the microbial utilization of thecae was expected to depend much more on the availability of external nutrient sources than the utilization of protoplasts. Indeed, decomposition of thecae did not occur in the absence of external nutrients but was rapid (1–2 d to their disappearance) after nutrients were added. In contrast, almost no stimulating effect of nutrient addition was observed for the decomposition of whole cells. The results suggest that intensive regenerative nutrient cycling or external nutrient inputs are a necessary precondition for an efficient trophic transfer of the energy stored in blooms of thecate dinoflagellates. The high nutrient demands of microbial degradation imply furthermore a competition for nutrients between heterotrophic degradative and phototrophic productive processes. Because of the generally assumed stronger competitive ability of heterotrophic bacteria, reduced primary production is expected as an indirect result of dinoflagellate bloom degradation. Indeed, reduced primary production is observed in Lake Kinneret every summer after the decline of the annual *Peridinium* bloom in June–July.

The seasonal plankton succession of the warm monomictic Lake Kinneret, Israel, is characterized by a typical winter–spring bloom formed almost exclusively by the armored dinoflagellate *P. gatunense* Nygaard (subsequently *Peridinium*). At peak development, *Peridinium* biomass usually exceeds 200 $g_{wet\ wt}\ m^{-2}$ and typically constitutes more than 90% of the estimated total phytoplankton biomass in the lake (Pollingher and Serruya 1976; Pollingher 1986). The bloom declines sharply in May–June, usually shortly after the establishment of thermal stratification, and is succeeded by other phytoplankton taxa the biomass of which, however, is considerably reduced.

As was shown by sediment trap studies conducted in Lake Kinneret (Zohary et al. 1998), in most years only a minor part of the biomass produced by *Peridinium* sedimented into the hypolimnion and bottom sediments, implying that the majority of the bloom biomass was decomposed or grazed within the epilimnion. Since grazing losses to the population of this large dinoflagellate are known to be minor (Serruya et al. 1980), these observations suggest that usually favorable preconditions for degradation of the bloom material exist in Lake Kinneret. However, Zohary et al. (1998) reported of exceptional years during which the amount of bloom material reaching the deep water layers was substantial. The observed interannual differences suggested also a potential impact of intrinsic factors (e.g., chemical structure of the organic material), external environmental factors (e.g., temperature, pH), or both on the actual rate of decomposition.

Among the many factors potentially influencing the rate of decomposition of algal material, a crucial role should conceptually be ascribed to the availability of nutrients. For thecate dinoflagellates, which are composed of two biologically and biochemically different main constituents (protoplasts and thecae), the role of nutrient availability has an added aspect. In *Peridinium*, the theca is a rigid shell consisting almost exclusively of polymeric carbohydrates (Loeblich 1969) and extremely deficient in N and P (C : N : P atomic ratio >3000 : 19 : 1; Zohary et al. 1998). The protoplast (ca. 60% of the cells' biomass) is the viable part of the organism, containing most of the proteins, nucleic acids, and storage compounds, and is relatively richer in nutrients (C : N : P of

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276 : 51 : 1) but were still P depleted in comparison with the Redfield ratio. Like many armored dinoflagellates, *Peridinium* sheds its theca during cell division (Pollingher 1988), thus generating during bloom development a continuous ''rain'' of C-rich but N- and P-poor particles. In addition to the natural division process, thecae may become shed by the cells under unfavorable environmental conditions. Overall, the biomass produced by *Peridinium* is comparatively carbon rich but P deficient, with a seasonal average C : P ratio of 460 : 1 (Wynne et al. 1982).

From this point of view, the food value of *Peridinium* is limited (Sterner and Hessen 1994), which in addition to the large cell size (40–50 μm diameter), may be a reason for its generally low direct utilization by grazers. It follows that most of the energy stored in the *Peridinium* bloom must be made available via detrital pathways involving conversion of C-rich and N and P-poor algal biomass into nutrient-enriched microbial carbon. However, because of the low nutrient contents, the availability of external nutrient sources must be considered as a necessary precondition for this process. Thus, a strong link between carbon and nutrient metabolism is to be postulated.

In order to better understand the impact of nutrients on the degradation process, we performed an experimental study with freeze-dried *Peridinium* cells (collected from the lake) and freeze-dried thecal material (from laboratory cultures, as it was impossible to collect the thecae from the lake). The role of external and internal nutrient sources was examined by comparing the rate of loss of dry weight for whole cells with that for thecae, with and without addition of nutrients. Concurrently, we followed the growth and enzymatic activities of the microbial community developing during the degradation process.

Methods

Peridinium material—Peridinium whole cells were collected from Lake Kinneret during the 1995 bloom with a 20-μm mesh net. Empty *Peridinium* thecae were collected from the material sedimenting to the bottom of the growth flask in axenic laboratory cultures of *Peridinium* isolated from Lake Kinneret and grown in Lindstom 16 medium (Lindström 1985). We have microscopic and enzymatic evidence from in situ decomposition experiments of natural *Peridinium* in dialysis bags suspended in Lake Kinneret (Hadas et al., unpubl. data) that in the presence of nutrients the decomposition of natural thecae was similar (in terms of rates, morphology, and microbial succession) to that observed in this study for culture-grown thecae. Both whole cells and thecae were washed with deionized water over a 20-μm mesh and freeze dried. Microscopic examination confirmed that the whole-cells material consisted of >95% *Peridinium* whole cells and that the thecae-only were indeed more than 95% empty thecae.

Incubation conditions—For the experiment, the lyophilized whole cells or thecae were resuspended in fresh, prefiltered (Whatman GF/C) subsurface lake water to a final dry weight concentration of 50 mg L^{-1} (corresponding roughly to the ambient concentrations in *Peridinium* patches) and incubated in duplicate 20-liter transparent bottles. For removal of externally adsorbed phosphate originating from the P-enriched growth medium without affecting structural P, the lyophilized thecae were washed in dilute (0.1 N) HCl and then washed three times in distilled water prior to resuspension in filtered lake water. In order to assure a sufficient inoculum of heterotrophic microbes, including bacteria and protozoa, 100 ml of 20-μm-filtered lake water was added to each bottle. The bottles were incubated in the dark at 20°C on a shaking table, aerated with air via a thin tube with its opening at the bottom of the bottle, and sampled for various determinations (listed below) at 1–3 day intervals. After 7 d of incubation, the remaining suspension in each 20-liter bottle was used to fill duplicate 2-liter transparent polycarbonate bottles (Nalgene). Nutrients, as 10 μM final concentration of NH_4Cl, 3 μM final concentration of KH_2PO_4, and metal mix at composition and final concentration as in the Lindström 16 medium, were added to one replicate of each pair of bottles. The bottles were incubated and sampled as described above for another 7 d. The experiment was terminated on day 11 because of apparent bottle effects (evaporative water loss), but microscopical examinations of the material continued until day 14.

Determinations

Dry weight (DW)—Determinations were performed on duplicate 100-ml subsamples from each bottle that were filtered through 47-mm GF/C filters and weighed after heating overnight at 70°C.

Microscopic observations—Peridinium live cells, dead cells, empty thecae, and naked protoplasts were identified microscopically as described by Zohary et al. (1998). The identification of bacteria, flagellates, and ciliates was made immediately on live samples. The degradation process and the development of a microbial degrading community were recorded photographically.

Microbial cell numbers—Bacterial and protozoan counts were determined on formalin (0.6%)-preserved samples by epifluorescence microscopy using 4′, 6-diamidino-2-phenylindole (DAPI) as a staining reagent (Güde et al. 1985).

Bacterial growth was estimated by measuring the incorporation rate of ^{14}C-leucine in triplicates (5-ml samples, final concentration 70 nM). A formalin-fixed sample served as control. The samples were incubated for 30–60 min at 20°C, filtered onto 0.45-μm Millipore filters, washed, and counted on the scintillation counter (Simon and Azam 1989).

Enzyme methyl-umbelliferyl (MUF) assays—For measurements of exoenzymatic activities, MUF substrates were used according to Chrost and Overbeck (1987). For the activity measurements, 5–10 ml of water samples were filtered through 0.4-μm polycarbonate filters. The filters were placed into petri dishes containing 4 ml of 0.1 M Tris buffer (pH 8.3–8.6), 40 μl of 0.1 M $MgCl_2$ solution, and 0.5 ml of 0.1 mM substrate solution (MUF-Phosphate, MUF α- and β-D-glucoside) and were incubated at 37°C for varying lengths of time (0.5–24 h). The enzyme reaction was stopped by

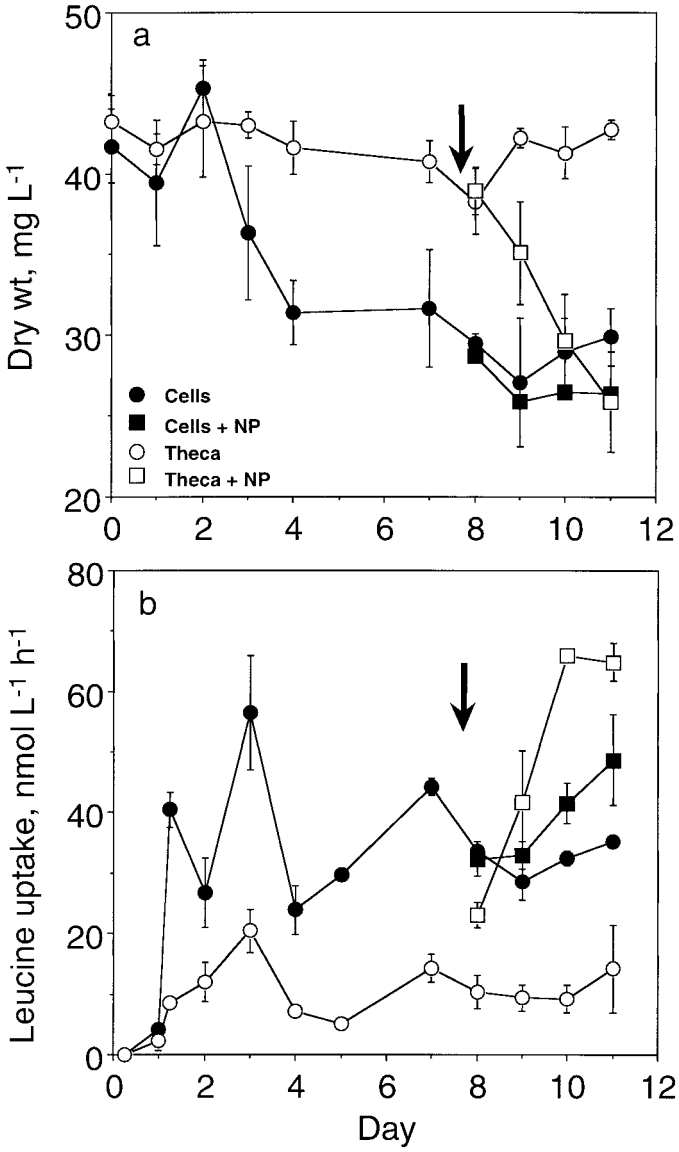

Fig. 1. Changes with time in (a) dry weight and (b) bacterial activity (measured as ^{14}C-leucine incorporation rates) during the decomposition of whole cells versus empty theca. Data are means for replicated treatments and bars are standard deviations (which do not show when smaller than the symbols). Arrows indicate the time when nutrients (N, P, metal mixture; indicated as ''+NP'') were added to half of the experimental bottles.

addition of 0.4 ml of 1 M NaOH, and the fluorescence was read in a fluorometer at an excitation of 365 nm and an emission of 460 nm. All measurements were performed on duplicate subsamples and calculated against a standard curve of MUF.

Polysaccharidase assays—For measurements of relative polysaccharidase activities (amylase, cellulase) 1 ml of water sample was added to 0.5 ml of remazol blue–stained substrate solution according to Wirth and Wolf (1992). After incubation at 37°C for 5 h, the reaction was stopped by addition of 0.5 ml 1 M HCl. The precipitated undecomposed polymeric material was separated by centrifugation, and the absorbance of the supernatant, containing the color-linked oligomeric and monomeric hydrolytic products of the enzyme activity, was measured spectrophotometrically in 1-cm cuvettes at a wavelength of 530 nm. The method allows only a measurement of relative activities. An extinction difference of 0.001 relative to the control was arbitrarily defined as one unit.

Table 1. Total dissolved and particulate N and P concentrations (μg L^{-1}) in: Lake Kinneret water used for resuspending the *Peridinium* cells and thecae, whole cells, and thecae-only experimental treatments, ca. 4 h after innoculation with *Peridinium* matter.

Water	TDN	Particulate N	TDP	Particulate P
Dilution water from Lake Kinneret, 1-m depth, 17 Mar 1996	590	240	4	16
Whole-cells treatment	865	1,725	76	60
Thecae-only treatment	415	125	5.5	11

Nutrient concentrations—Orthophosphate, total dissolved phosphate, total phosphate, nitrate, ammonia, and total nitrogen contained in 0.45-μm-filtered and in unfiltered subsamples were determined according to standard methods (American Public Health Association 1992) by the chemical laboratory of the Watershed Unit, Mekorot Water Company.

Results

Initial loss of DW—In all experimental bottles, initially measured DWs ranged generally 15–25% below the theoretically expected values of 50 $mg_{dry\ wt}$ L^{-1} (Fig. 1a). This indicates that prior to microbial degradation, part of the bloom material became solubilized because of abiotic leaching. This was also demonstrated by the concentrations of dissolved nitrogen and phosphorus in the whole-cells treatments, which at the start of the experiment (4 h after the addition of *Peridinium* material to the lake water) exceeded the values of Lake Kinneret water considerably (Fig. 2c,d, Table 1).

Degradation patterns without nutrient addition: whole cells—In the whole-cells treatment, DW declined initially more or less continuously, following a linear decay pattern. Without the addition of nutrients, roughly 50% of the initial DW was lost during the 10 d of incubation. Microscopic examination of the whole-cells treatment samples revealed that generally the degradation of the enveloping thecae preceded that of the protoplasts, many of which remained intact until the end of the experiment. Provided enough nutrients were available (see below), within 3 d of incubation cellular thecae disappeared completely (i.e., decayed to a level where they could not be identified microscopically as thecae), leaving behind naked protoplasts. Repeated microscopic observation of the same individual theca showed that it became microscopically unrecognizable within 24 h.

The degradation of the whole cells was accompanied by

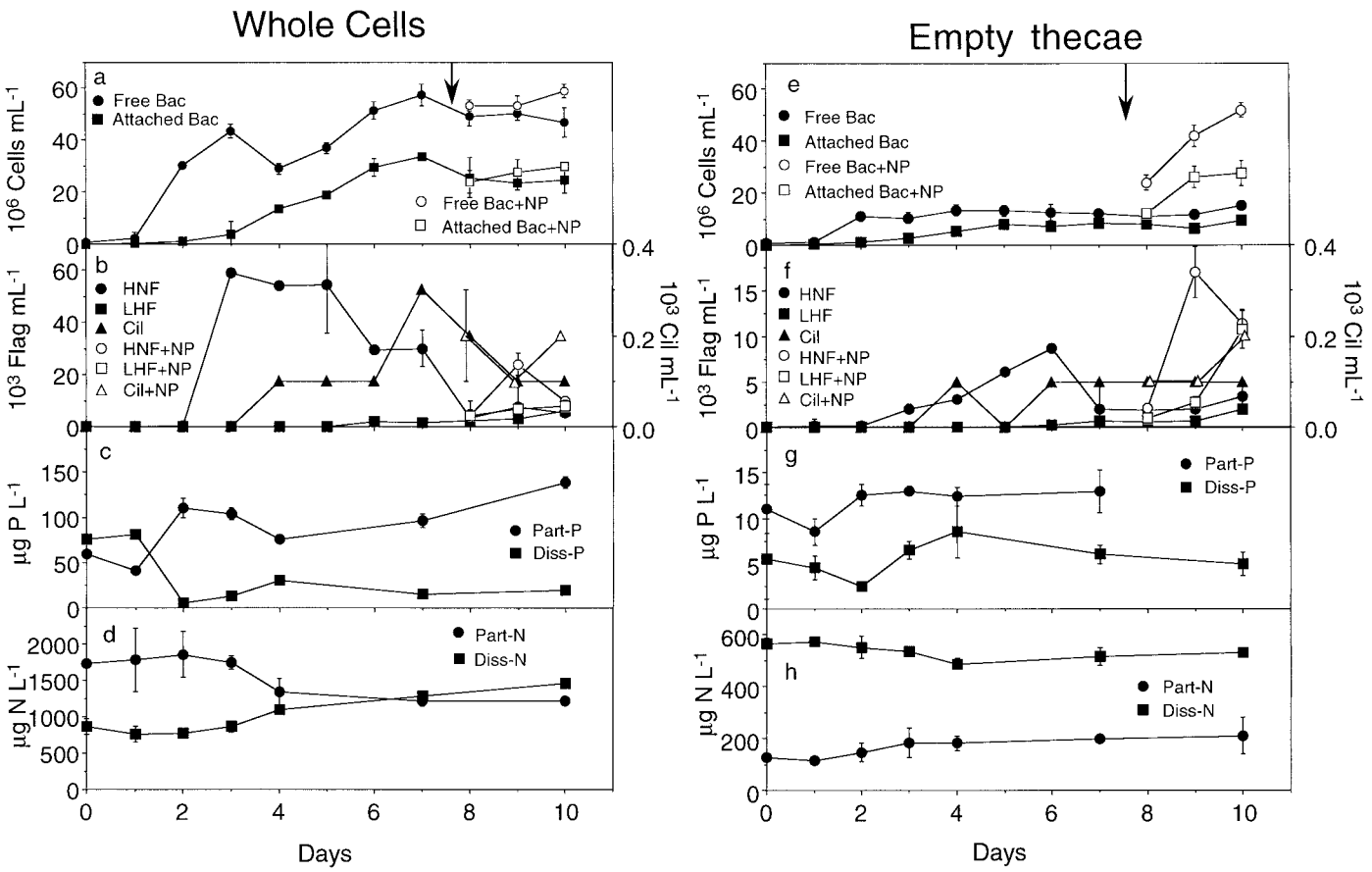

Fig. 2. Changes with time in abundances of free and attached bacteria, protozoa, and N and P concentrations during the decomposition of *Peridinium* whole cells versus empty thecae. Arrows indicate the time when nutrients (N + P + metal mixture; indicated as "+NP") were added to half of the experimental bottles. Data are means for replicated treatments and bars are standard deviations (which do not show when smaller than the symbols). Full symbols indicate unenriched treatments; empty symbols indicate after nutrient addition. Flag = flagellates (HNF—heterotrophic nanoflagellates; LHF—larger heterotrophic nanoflagellates); CIL = ciliates (note the second y-axis for ciliates).

intensive development of heterotrophic microbial populations. These consisted initially exclusively of rod-shaped motile bacteria that were growing as free, dispersed single cells and reached maximum densities of ca. 45×10^6 cells ml^{-1} after 3 d of incubation (Fig. 2a). The observed rapid bacterial growth became further apparent by steep increases of leucine uptake rates that peaked after 2–3 d at values ranging between 30 and 60 nmol L^{-1} h^{-1} (Fig. 1b). With a time lag of ca. 24 h, the bacterial maxima were followed by intensive development of bacterivorous heterotrophic nanoflagellates (HNF), belonging mostly to the genus *Spumella*, which reached numbers up to $50–60 \times 10^3$ cells ml^{-1}. The increase of flagellates was followed by a drop in the density of free bacteria and in leucine incorporation rates from day 3 to day 4.

Simultaneously with the mass appearance of protozoans in the whole-cells treatments, the structure of the bacterial community changed, from dominance of free forms to increasing proportion of attached forms (Fig. 2a). Among the attached bacteria, filamentous forms were conspicuous although numerically infrequent relative to small rods and cocci. Later in the succession populations of larger, heterotrophic flagellates (LHF) and of flagellates growing attached to the detrital material (e.g., *Rhynchomonas* and *Bodo*) were observed. Finally, free-swimming ciliates appeared, although they appeared only at low densities of up to 300 ml^{-1}. A recovery in densities of free bacteria and a continuous increase in the numbers of attached bacteria was observed between days 4 and 7. During the later stages, from day 7 to the end of the experiment, bacterial abundances and leucine incorporation rates in the whole-cells treatment without nutrient addition were maintained at a relatively high level. Fungal growth was not observed during the entire experiment.

The total amounts of nitrogen, TN, and phosphorus, TP, in the whole-cells experimental bottles were approximately 2,500 μg N L^{-1} and 135 μg P L^{-1} (Fig. 2c,d; Table 1). This is by far more than the total amount of nutrients contained in the added Lake Kinneret water (Table 1). Evidently, more than 90% of the nutrients originated from the lyophilized bloom material. Out of these total amounts, a substantial fraction was initially in the dissolved form because of the above-mentioned leaching. Coinciding with the mass development of bacteria by the second day, part of the dissolved

Table 2. Activities of various enzymes (mean ± standard deviation for duplicated treatments) at the onset (day 0) and along the decomposition process (day 2 and day 10) of *Peridinium* whole cells and empty thecae. Nutrients (as NH_4, PO_4^{-3}, and trace metals) were added on day 7 to the ''+ nutrients'' treatments. Units for amylase and cellulase are given as relative optical density (OD) values, where an extinction difference of 0.001 relative to the control was arbitrarily defined as one unit.

Treatment	Day	α-Glucosidase (nmol MU L^{-1} h^{-1})	β-Glucosidase (nmol MU L^{-1} h^{-1})	APA (nmol MU L^{-1} h^{-1})	Amylase (relative OD units)	Cellulase (relative OD units)
Whole cells	0	0.35 ± 0.1	11.8 ± 1.0	26 ± 4.2	2.6 ± 2.5	3.3 ± 0.8
Whole cells	2	5.2 ± 0.07	4.3 ± 0.3	3,799 ± 429	90.2 ± 20.7	2.2 ± 1.1
Whole cells	10	22.4 ± 2.4	52.0 ± 6.6	3,788 ± 356	58.7 ± 14.0	10.4 ± 3.3
Whole cells + nutrients	10	41.9 ± 4.8	76.1 ± 4.7	2,304 ± 83	80.8 ± 15.4	44.4 ± 23.9
Thecae	0	1.05 ± 0.2	9.4 ± 0.1	14.5 ± 0.7	7.0 ± 5.6	4.9 ± 1.2
Thecae	2	1.95 ± 0.2	1.9 ± 0.1	2,443 ± 228	37.8 ± 6.2	1.6 ± 0.9
Thecae	10	26.1 ± 8.6	55.0 ± 19.0	3,432 ± 303	40.6 ± 10.4	0.6 ± 0.5
Thecae + nutrients	10	136 ± 78	170 ± 37	2,630 ± 530	46.2 ± 16.3	51.3 ± 3.8

N and almost 100% of the dissolved P were converted into particulate P (Fig. 2c,d). After the beginning of microbial growth, most of the TP and substantial parts of the TN remained bound in the particulate fraction, presumably because of incorporation by living heterotrophic biomass of the microbial loop organisms. However, coinciding with the mass development of bacterivorous grazers, the dissolved fraction of P and N increased again, indicating a release of nutrients from decomposing *Peridinium* biomass and regeneration of nutrients by the protozoan bactivors (Hadas et al. 1990). The percentage of dissolved P decreased again from day 4 to 7.

The initial steep increase of bacterial abundances was accompanied by a ca. 150-fold increase of alkaline phosphatase activity over the first 2 d (Table 2), suggesting the development of P-deficient conditions that require the induction of alkaline phosphatase activity, and in agreement with the observed depletion of dissolved P forms in the same bottles. Concurrently, amylase activity increased ca. 30-fold. The activity of α-D-glucosidase increased gradually with time, reaching on day 10, without nutrient addition, a value that was ca. 60-fold higher than at the start of the experiment. A less steep increase in values over time was recorded for β-D-glucosidase. Cellulase activities remained almost undetectable during the fist week, after which an increase was observed.

Degradation patterns without nutrient addition: empty thecae—A rather different degradation pattern was observed for the empty thecae experimental treatment (Fig. 1, 2). The most striking difference was that practically no loss of DW was observed during 11 d of observation in the absence of external nutrients. Consistent with this apparent lack of degradation, the bacterial and protozoan populations and activities developed to a much lesser extent than in the corresponding whole-cells treatment: bacterial and protozoan densities reached only 25% and 20%, respectively, of those observed in the whole-cells treatment, and leucine incorporation rates reached values of about 25% of those observed with whole cells. However, similar to the pattern for whole cells, the pattern of bacteria → protozoa succession was characterized by an initial increase of free dispersed bacteria that was followed by increasing proportion of attached and filamentous bacteria after the increase in the abundance of grazing protozoa.

Corresponding to the low initial nutrient concentration in the empty thecae treatment (Table 1), throughout the experiment, TN and TP concentrations remained at considerably lower levels then in the whole-cells treatment (Fig. 2g,h). Also, the observed dynamics for the dissolved and particulate fractions were substantially less expressed. Nevertheless, even in the empty thecae treatment, the initial conversion of dissolved nutrients into microbial particulate biomass and the partial release of nutrients coinciding with protozoan development could be observed.

Without the addition of nutrients, measured enzyme activities were mostly similar or lower than those observed with whole cells (Table 2). Microscopic examination confirmed that in the empty thecae treatment with no nutrient addition, the thecae remained intact throughout the experiment and that the microbial colonization of the thecae was limited compared to that observed for whole cells.

Effect of nutrient addition—The addition of nutrients to the whole-cells treatments had no dramatic effects on DW losses (Fig. 1a), although slight increases of bacterial and protozoan numbers (Fig. 2a,b) and of some enzymatic activities (Table 2) were recorded. In contrast, pronounced effects were observed in the empty thecae treatments, where the addition of nutrients led to the loss of 33% of the DW over 3 d in comparison with practically no change in DW without nutrient addition (Fig. 1a). Similarly, a 3-fold increase of leucine incorporation rates from day 8 to day 10 was observed only when nutrients were added (Fig. 1b). Concurrently, bacterial and protozoan abundances responded to nutrient addition with sharp increases (Fig. 2e,f). Furthermore, nutrient addition to thecae-only bottles resulted with much higher activities for most enzymes (3- to 83-fold higher, depending on the enzyme) relative to thecae-only bottles without nutrient addition (Table 2). Notably, phosphatase activity was reduced by the addition of nutrients. This was the expected outcome of the relaxation of the demand for P.

The dramatic effect of nutrient addition is summarized (Fig. 3) by plotting for each parameter the ratio of the values for the ''nutrients added'' to the ''no nutrients added'' treatments of the same material (whole cells; thecae). The values

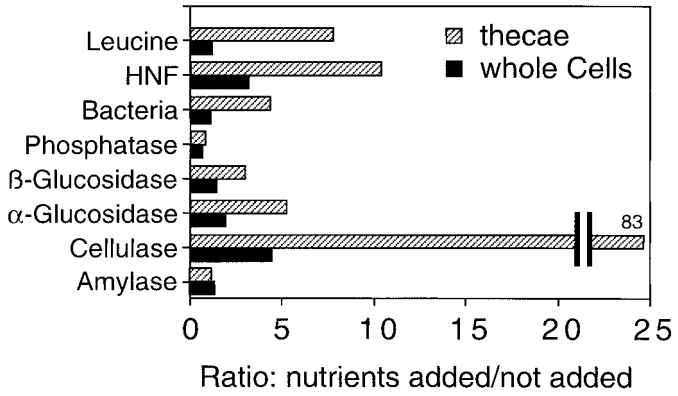

Fig. 3. The effect of nutrient addition on a range of microbial and enzymatic parameters during the decomposition of *Peridinium* whole cells and thecae. This effect is presented as the ratio of the values for the ''nutrients added'' to the ''no nutrients added'' treatments, on day 10, i.e., 3 d after nutrients were added to half of the bottles.

used for computing the ratio were those measured on day 10, i.e., 3 d after nutrients were added to half of the bottles. The effect was most pronounced for the structural polysaccharidase, cellulase, which remained almost undetectable unless nutrients were added. The decreased activity in the case of phosphatases in response to nutrient addition was also apparent, as expressed by a ratio <1.

Microscopic examination revealed that the microbial colonization of thecae increased tremendously after the addition of nutrients. Among the colonizing bacteria, gliding bacteria belonging to the Cytophaga group were dominant. Following the increased microbial colonization, a progressive deterioration and thinning of the thecal structures became visible microscopically within a short time.

Discussion

The succession of microbial communities together with the dynamics of the chemical parameters observed in these experiments is similar to that frequently reported for degradation experiments using natural plant material (Jürgens and Güde 1994). In those studies, the initial bacterial growth was generally shown to be mainly because of utilization of the dissolved organic carbon sources and inorganic or organic nutrients leaching out of the plant cells. Only later particulate carbon sources were utilized, coinciding with an intensified microbial colonization of the particulate material. This pattern was observed also in the present study. It was reflected by the course of enzymatic activities associated with the degradation of structural polysaccharides and confirmed by microscopic observations.

Assuming a conversion factor of 2 μg C per nM leucine incorporated (Simon and Azam 1989), a cumulative bacterial production in the range of 60–120 μgC L^{-1} h^{-1} would be estimated for the initial 3 d in the whole-cells treatment. This would mean that 3–6 mg C L^{-1}, or roughly 12–25% of the added *Peridinium* carbon, would have been converted into bacterial biomass. This estimate is approximately that estimated to be solubilized by abiotic leaching at the onset of the experiment. In fact, the absolute amount of carbon utilized by bacteria should be about double, assuming that ca. 50% of the carbon utilized by Lake Kinneret bacteria is respired (Cavari et al. 1978). This rough estimate supports the assumption that the pool of dissolved carbon was exploited within the first few days and that later further microbial growth could only occur by exploitation of particulate structural carbon sources.

The observed pattern of enzyme activities associated with the utilization of low and high molecular carbon can be seen as a further confirmation of a preferential utilization of low molecular and dissolved organic material during the initial phase. The activities of enzymes participating in the degradation of the particulate organic material (above all cellulase, but also β-glucosidase) were delayed until extracellular dissolved material was consumed. Such sequential degradation patterns are frequently observed in similar experimental systems (Chrost 1990). The enzyme assays conducted in the present study, in which only V_{max} was measured, provide primarily information on degradation potentials and not real degradation rates. Nevertheless, such assays can be taken as indicators for the predominant substrate utilization pathway at the different experimental treatments.

The regularly observed shift from free to complex and attached growth forms colonizing the particulate surfaces, coinciding with the mass development of bactivorous protozoans, seems to support additionally the intensified use of particulate matter after exhaustion of the labile dissolved matter. It was beyond the scope of this study to identify the taxonomic identity of the bacterial groups colonizing the algal material. However, the observed growth of attached gliding bacteria belonging to the *Cytophaga* group can be seen as a further confirmation of intensified polysaccharide degradation, because these bacteria are known as efficient utilizers of structural carbon under aerobic conditions (Christensen 1977; Amann et al. 1995).

Besides the exhaustion of dissolved substrates, the development of complex growth forms combined with the attachment to surfaces could be stimulated by increasing grazing pressure exerted selectively on free dispersed bacteria by protozoa (Jürgens and Güde 1994). Also in the presented experiments, the shift from free to attached (more grazing resistant) growth forms coincided with the increase in protozoa following the initial bacterial maximum. Other than bacteria and protozoa, no microbial populations could be identified in these experiments. In similar *Peridinium* degradation experiments reported elsewhere (Zohary et al. in press), fungi were occasionally observed. Although it remains unclear to what extent these fungi were involved in the process of decomposition, the existing evidence suggests that bacterial rather than fungal populations were the predominant primary decomposers of *Peridinium* blooms.

As was confirmed by the analysis of the elementary composition, thecae contained much less dissolved and particular nitrogen and phosphorus than the whole cells (Zohary et al. 1998). However, even the whole-cell C : P ratios of $>400:1$ fall far above the ratios of 40–50 : 1 measured for bacterioplankton (Jürgens and Güde 1990). It follows that microbes growing on this material will suffer from more (thecae) or less (whole cells) severe shortage of nutrients. Therefore

high fluxes of nutrients are required for an efficient microbial degradation of *Peridinium.* This may occur either by intensive recycling of nutrients (e.g., because of intensive grazing) or by use of an external source of nutrients.

As was demonstrated for the whole cells, protozoan grazing led indeed to a liberation of nutrients from biomass. This can be interpreted as efficient P and N regeneration by the grazers, as is often reported (Anderson 1997). Therefore, grazers reduced the degree of nutrient limitation for bacterial growth. Although the percentage of dissolved P decreased again from day 4 to 7 (possibly because of increased P binding by grazing-resistant bacteria) it must be assumed that P regeneration by grazers remained relatively efficient because addition of nutrients caused only a slight stimulation of bacterial activities in the whole-cells treatment as compared with the effects observed for microbial populations growing on the P-deficient thecae. Consequently, the addition of nutrients had only little effect on the degradation pattern of the whole cells. In contrast, marked effects on the rate of degradation were observed for the empty thecae, where the total amount of nutrients present initially was by far lower than in the whole cells so that recycling by protozoans was insufficient to meet the high demand for nutrients required for the breakdown of the carbon rich material. With the addition of nutrients, the limitation was relaxed and degradation processes could proceed.

Assuming molar C : N : P ratios of 40 : 6 : 1 for bacteria (Jürgens and Güde 1990), the estimated cumulative bacterial net production of 3–6 mg C L^{-1} during the first 3 d would have resulted in P demands of ca. 200–400 μg P L^{-1}, exceeding the available total phosphorus roughly twofold, while the corresponding nitrogen demands (500–1,000 μg NL^{-1}) were covered by the nitrogen present in the system (>2,000 μg NL^{-1}). Thus, the observed shift from dissolved to particulate P was almost completely because of bacterial P incorporation. Moreover, it is suggested that bacterial growth was P limited at that time rather than C or N limited.

Our results suggest that the annual *Peridinium* bloom in Lake Kinneret is subject to comparatively rapid degradation, provided that nutrients are available. We have shown that in the presence of nutrients, thecae degradation is rapid and precedes that of protoplasts. Hertzig et al. (1981) and Sherr et al. (1982) also noted that *Peridinium* thecae disappeared faster than the protoplasts, but they did not identify the prerequisite of presence of nutrients. The relative recalcitrance of the protoplasts seems to be caused by intrinsic structural properties (i.e., sporopolleninlike membranes; cf. Gunnison and Alexander 1975) rather than by unfavorable external factors. In spite of the high C : P ratios of the bloom material and the generally low external nutrient concentrations occurring in epilimnetic Lake Kinneret water layers, the degradation of *Peridinium* protoplasts seems usually not to be limited by nutrient availability. This suggests that either internal phosphorus regeneration (mainly via grazing) or external P supply (via river loads or mobilization of littoral P deposits by wind-induced resuspension) are usually supplying the P demands in Lake Kinneret.

At the same time, our results suggest that when nutrients are insufficient, *Peridinium* thecae decomposition would be slowed down considerably. This is in accordance with observations by Zohary et al. (1998), that during 1991, a low-inflow drought year, sedimentation losses of *Peridinium* in Lake Kinneret, and especially those of thecae, were much higher than during the normal and high-inflow years of 1992–1994. Possible explanations provided were that in 1991 decomposition rates were lower and that resuspension rates of undecomposed thecae were higher. The results reported in this paper suggest that nutrient limitation leading to delayed degradation may have been at least partially responsible for the observed high 1991 thecal sedimentation rates. Although at present it cannot be decided whether inefficient nutrient regeneration, reduced external P supply, or both were causing the P limitation, there are at least hints for a potential role of changing P loads. These were significantly lower in 1991, when the delayed thecal decomposition was observed, than in other years (Zohary et al. 1998).

An important, more general conclusion is that, in P-limited stratified lake nutrients (N and P) contained in dinoflagellate protoplasts are likely to be effectively recycled within the epilimnion, while the C-rich and N- and P-poor thecae are more likely to reach the bottom sediments. A similar phenomenon of selective C sedimentation is the mass sedimentation of *Phaeocystis*-derived organic matter, mostly C-rich and N-deficient mucilage, in the Barents and Northern Seas (Wassmann et al. 1990; Riebesell et al. 1995). It is interesting to note that N depletion of the thecae was even more severe than its P depletion: protoplasts contained 10 times more P but also 27 times more N than thecae. In the marine environment, where N is usually the limiting nutrient, the extreme N depletion of dinoflagellate thecae could be more crucial for nutrient cycling.

Overall, the demonstrated high nutrient demand required for bloom degradation implies a significant competition between autotrophs and heterotrophs. As a consequence, primary producers should become also sharply nutrient limited during the bloom degradation phase. In this way, the bloom could have a prolonged echo in the lake even after its disappearance. Therefore, the *Peridinium* bloom may decrease the channeling of photosynthetic carbon to higher trophic levels directly because of its low ingestibility by zooplankton grazers and indirectly because of high nutrient binding during degradation. Conversely, the availability of external or internal nutrients should also have a strong impact on the transfer of energy to higher levels.

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