

# Changes in Bacterial and Eukaryotic Community Structure after Mass Lysis of Filamentous Cyanobacteria Associated with Viruses†

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**During an experiment in two laboratory-scale enclosures filled with lake water (130 liters each) we noticed the almost-complete lysis of the cyanobacterial population. Based on electron microscopic observations of viral particles inside cyanobacterial filaments and counts of virus-like particles, we concluded that a viral lysis of the filamentous cyanobacteria had taken place. Denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA fragments qualitatively monitored the removal of the cyanobacterial species from the community and the appearance of newly emerging bacterial species. The majority of these bacteria were related to the *Cytophagales* and actinomycetes, bacterial divisions known to contain species capable of degrading complex organic molecules. A few days after the cyanobacteria started to lyse, a rotifer species became dominant in the DGGE profile of the eukaryotic community. Since rotifers play an important role in the carbon transfer between the microbial loop and higher trophic levels, these observations confirm the role of viruses in channeling carbon through food webs. Multidimensional scaling analysis of the DGGE profiles showed large changes in the structures of both the bacterial and eukaryotic communities at the time of lysis. These changes were remarkably similar in the two enclosures, indicating that such community structure changes are not random but occur according to a fixed pattern. Our findings strongly support the idea that viruses can structure microbial communities.**

Photosynthetically derived organic carbon is one of the major energy sources for heterotrophic bacteria in oceans and lakes (3, 6, 16). This organic carbon is made available to the bacteria through various pathways. Exudation by phototrophs and excretion by their grazers provide rather constant release of carbon. The decline of phytoplankton blooms releases dissolved organic carbon in a short time, which can be rapidly used by heterotrophic bacteria (4, 13, 20, 51). To study the growth of heterotrophic bacteria on exudates released by cyanobacteria, we conducted experiments in laboratory-scale enclosures (LSEs) filled with lake water. However, 15 days after the experiments started nearly all filamentous cyanobacteria lysed. Electron microscopic observations of viruses inside filaments of cyanobacteria and counts of virus-like particles indicated a viral lysis event.

Cell lysis is a major cause of phytoplankton bloom decline (13, 51), and many studies have shown the importance of viruses in phytoplankton mortality (12, 20, 44, 48, 49). These viruses are considered to be important members of the microbial loop (5, 8, 49). High viral abundance and decay rates suggest considerable viral activity (10), which is also indicated by observations of microbial cells containing mature viral particles (42). It has been calculated that 10 to 20% of the marine bacterial community is lysed by viruses on a daily basis (47). Approximately the same mortality was found in a freshwater study (23). Hence, viral lysis is a significant factor in controlling

bacterial and primary production (23, 33, 49, 54) and carbon and nutrient flow within the microbial loop (9, 34).

Besides controlling carbon production, viruses are also thought to structure microbial communities (25). Similar to the size-selective grazing of bacterivores (30), viral host specificity could be a very strong structuring force of microbial communities. The lysis and removal of species from the microbial community and the consecutive nutrient release may give other species the opportunity to proliferate. To test the hypothesis that viruses could structure the microbial community, we used denaturing gradient gel electrophoresis (DGGE) (19) to follow the changes in the structure of both the bacterial and eukaryotic communities before and after the lysis event. DGGE analysis of 16S and 18S ribosomal DNA (rDNA) fragments circumvents the problem of underestimating microbial diversity due to noncultivable microorganisms. This molecular technique has been used extensively to profile natural bacterial diversity (18, 36, 50), and statistical analysis of the DGGE patterns can reveal relative changes in the microbial community structure (52, 53).

## MATERIALS AND METHODS

**Experimental design.** Two LSEs, especially designed to mimic the physical environment of Lake Loosdrecht, The Netherlands (46), were each filled with 130 liters of Lake Loosdrecht water sampled on 26 November 1996. Lake Loosdrecht is a shallow eutrophic lake dominated by filamentous cyanobacteria. The water temperature at the time of sampling was 3.6°C. The temperature was raised to 20°C within 1 day. Both LSEs were supplied with medium at a dilution rate of 0.05 day<sup>-1</sup>. The incident irradiance was 50 W · m<sup>-2</sup> during a 16-h light period. The LSEs were stirred continuously to assure complete mixing. After 1 week of adaptation, both LSEs received elevated light levels of 150 W · m<sup>-2</sup> during 4 h around the midpoint of the light period. In one system (LSE 1), stirring was halted during this high-light period. Elevated light levels were used to trigger exudate production.

**Chl-*a*, virus-like particles, and numbers of bacteria.** Chlorophyll *a* (Chl-*a*) concentrations were measured after hot-ethanol extraction (35). Virus-like par-

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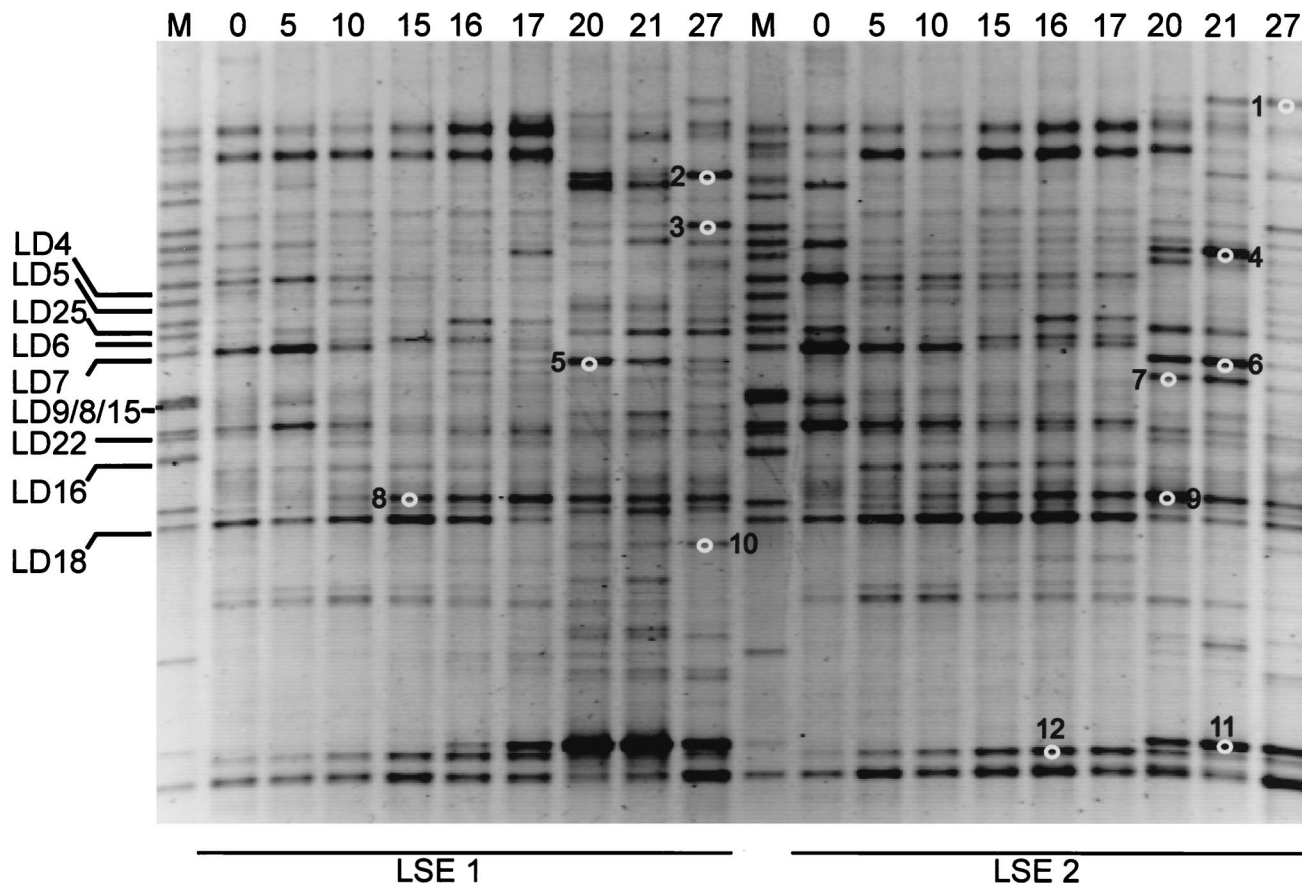


FIG. 1. Negative image of an ethidium bromide-stained DGGE pattern of the bacterial community structures of LSEs 1 and 2. The numbers at the top of the image indicate days from the start of the experiment. The numbered white circles refer to the excised and sequenced bands explained in Table 3. Lane M, marker lane containing the clones from the Lake Loosdrecht clone library. Clones related to cyanobacteria are indicated by "LD" and are elucidated in Table 2.

to cyanobacteria that could be detected. Until day 15, the number of sequence types as detected by DGGE remained almost constant (Fig. 2). After the lysis event (day 15), many new sequence types were appearing in the DGGE patterns of both LSEs. Twelve of these new bands were excised from the gel and sequenced to obtain phylogenetic information. Four of the bands (bands 1, 2, 4, and 7) appeared to be related to the *Cytophagales*, three (bands 3, 5, and 6) appeared to be related to the  $\beta$ -*Proteobacteria*, three (bands

10, 11, and 12) appeared to be related to the actinomycetes, and two (bands 8 and 9) appeared to be related to the  $\alpha$ -*Proteobacteria* (Table 3). Analysis of the DGGE patterns by NMDS (Fig. 3) showed a relatively constant bacterial community structure for LSE 1 until day 15. From day 15 to day 20, the community structure showed large changes. Thereafter, changes were relatively small again. The community structure of LSE 2 remained constant until day 17, and then a major change occurred between days 17 and 20.

TABLE 2. Sequence similarities of the cyanobacterial clones used as marker bands in Fig. 1 (lane M)<sup>a</sup>

Designation	Clone		Species	Closest relative	
	EMBL accession no.	% Similarity		EMBL accession no.	Taxonomic description
LD4	AJ006279	95.8	<i>O. limnetica</i>	AJ007908	Filamentous cyanobacterium
LD5	AJ007865	91.5	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium
LD6	AJ006280	99.5	<i>O. limnetica</i>	AJ007908	Filamentous cyanobacterium
LD7	AJ007864	93.0	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium
LD8	AJ006281	94.3	<i>Nodularia</i> sp.	AJ224447	Filamentous cyanobacterium
LD9	AJ006282	89.2	<i>Synechococcus</i> sp. strain PCC 6301	AF001477	Cocoid cyanobacterium
LD15	AJ006283	91.1	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium
LD16	AJ007866	98.9	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium
LD18	AJ006284	99.8	<i>O. agardhii</i>	X84811	Filamentous cyanobacterium
LD22	AJ006285	99.8	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium
LD25	AJ006286	90.6	<i>P. hollandica</i>	AJ007907	Filamentous cyanobacterium

<sup>a</sup> Sequences were aligned to the closest relative from the EMBL database. The similarity was calculated with gaps and ambiguities not taken into account.

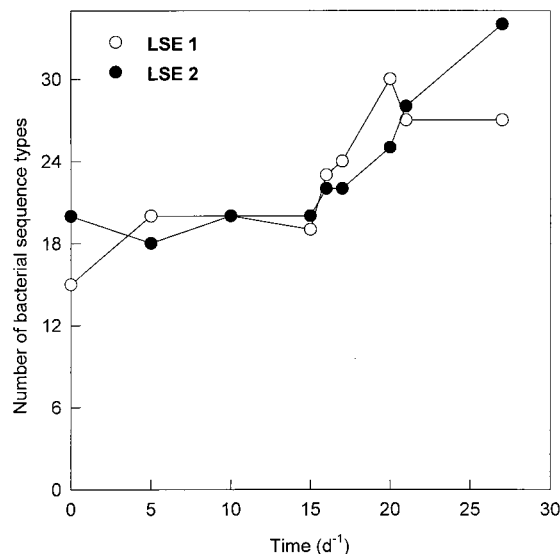


FIG. 2. Numbers of bacterial sequence types detected by DGGE analysis during the course of the experiment.

During the last 7 days, changes in the community structure were relatively small again.

**Eukaryotic community structure.** The DGGE analysis of the 18S rDNA fragments of both LSEs showed no notable change in the banding pattern until day 17. The DGGE patterns of both LSEs on day 20, and the DGGE pattern of LSE 2 on day 21, were completely dominated by a single band (Fig. 4). Excision and sequence analysis showed that this band (excised bands 6 and 7) represented a rotifer-like sequence. Rotifer abundance was estimated on days 17, 20, 21, and 27. In LSE 1 the densities were  $1 \times 10^4$ ,  $3.1 \times 10^4$ ,  $1.7 \times 10^4$ , and  $0.1 \times 10^4$  individuals  $\cdot$  liter $^{-1}$ , respectively. In LSE2 the numbers were  $0.1 \times 10^4$ ,  $4 \times 10^4$ ,  $7 \times 10^4$ , and  $0.8 \times 10^4$  individuals  $\cdot$  liter $^{-1}$ , respectively. Other dominant bands that appeared after the lysis event were excised from the gel and sequenced. Their similarities to the closest sequences in the EMBL database are shown in Table 4. Three of these bands (1, 3, and 4) showed

TABLE 3. Sequence similarities of the excised bacterial bands that appear in Fig. 1<sup>a</sup>

Band No.	% Similarity	Closest relative		
		Species	Accession no.	Taxonomic description
1	96.5	<i>Flavobacterium</i> sp.	U63936	Cytophagales
2	86.6	<i>Flexibacter sancti</i>	M62795	Cytophagales
3	97.4	Unidentified bacterium LD28	Z99999	$\beta$ -Proteobacteria
4	97.4	<i>Flavobacterium</i> sp.	U63936	Cytophagales
5	98.8	<i>Blastobacter</i> sp.	U20772	$\beta$ -Proteobacteria
5	98.8	Unidentified bacterium	AJ223452	$\beta$ -Proteobacteria
6	100	<i>Blastobacter</i> sp.	U20772	$\beta$ -Proteobacteria
6	100	Unidentified bacterium	AJ223452	$\beta$ -Proteobacteria
7	96.8	<i>Flavobacterium aquatile</i>	M62797	Cytophagales
8a	100	<i>Sphingomonas adhaesiva</i>	X72720	$\beta$ -Proteobacteria
8b	96.4	<i>Hyphomicrobium vulgare</i>	X53182	$\alpha$ -Proteobacteria
9	96.4	<i>Hyphomicrobium vulgare</i>	X53182	$\alpha$ -Proteobacteria
10	98.3	Unidentified bacterium	U85190	Actinomycetes
11	97.1	Unidentified bacterium	U85190	Actinomycetes
12	99.4	<i>Mycobacterium</i> sp.	U46146	Actinomycetes

<sup>a</sup> Sequences were aligned to the closest relatives from the EMBL database. The similarity was calculated with gaps and ambiguities not taken into account.

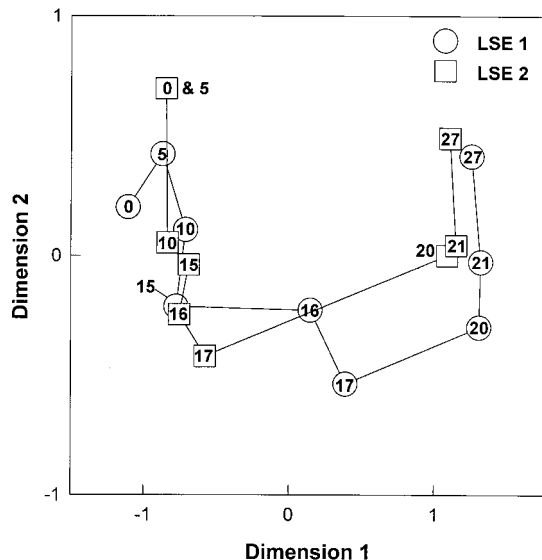


FIG. 3. NMDS map showing the changes in the structures of the bacterial communities during the lysis event. The numbers inside the symbols refer to the days of the experiment.

very low similarity (<90%) to known eukaryotic sequences. Band 2 was related to the Ciliophora. Bands 6, 7, and 9 were very closely related (>99%) to the rotifer *Brachionus plicatilis*, and band 8 was related to an Arthropoda sequence.

Analysis of the eukaryotic DGGE patterns by NMDS revealed that only small changes occurred in the eukaryotic microbial community during the first 20 days of the experiment (Fig. 5). Five days after the lysis event a major change in the eukaryotic communities of both LSEs was observed. Thereafter, the community structure returned to a state approximately similar to that preceding the lysis event.

## DISCUSSION

**Quantitative versus qualitative DGGE.** The PCR-based methods we used in this study merely give a qualitative view of the changes in both the bacterial and eukaryotic community structures after the viral lysis event. Many studies have shown that these methods are prone to give a quantitatively incorrect view of the microbial community (17, 22, 27, 39, 45). An example is the apparently increasing dominance of the *Oscillatoria agardhii*-related band (LD18) during the first 16 days of the experiment. Microscopic estimations revealed that *O. agardhii* contributed less than 1% to the total cyanobacterial biomass. Another example is the complete dominance of the eukaryotic DGGE patterns by a single rotifer band. Since rotifers are metazoa, every individual contributes many cells, and thus many copies of their rRNA gene, to the PCR. Unless multicellular species can be removed from the sample, eukaryotic DGGE patterns have to be interpreted cautiously. To exclude these errors, we did not use the intensities of the DGGE bands as a measure of abundance. Instead, we reduced the information to simply presence or absence of a sequence type. Thus, only the complete removal or new emergence of sequence types would be detected by NMDS.

**Viruses as structuring forces.** We started an experiment to follow the growth of heterotrophic bacteria on exudates of cyanobacteria. However, all filamentous cyanobacteria lysed, and we were able to monitor the appearance of previously undetected bacterial and eukaryotic species on the released

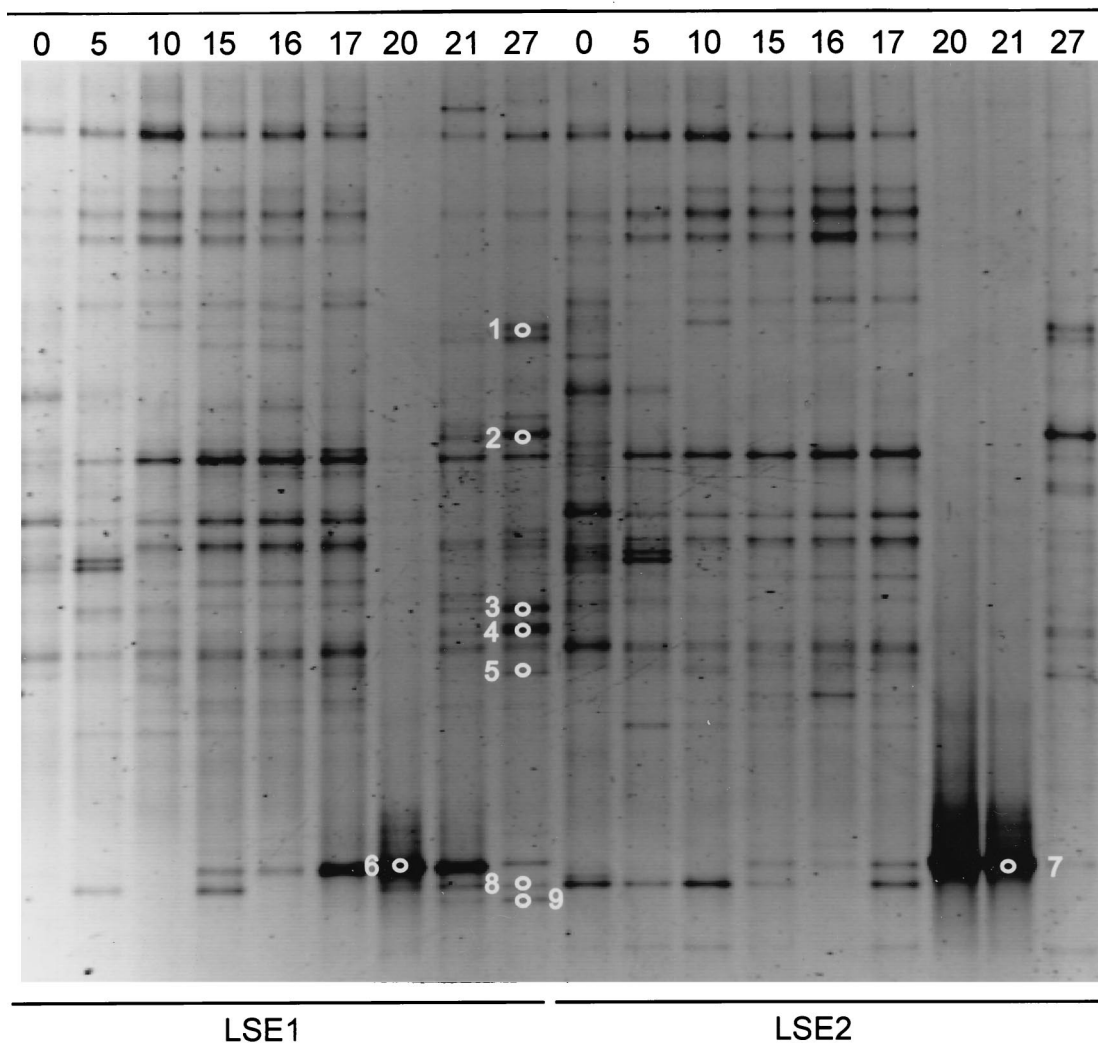


FIG. 4. Negative image of an ethidium bromide-stained DGGE gel of the eukaryotic community structures of LSEs 1 and 2. The numbers at the top of the image indicate the days from the start of the experiment. The numbered white circles refer to the excised and sequenced bands elucidated in Table 4.

carbon. From counts of virus-like particles and observations of numerous free virus particles and viruses attached to filaments of cyanobacteria, we concluded that there had been a massive viral outbreak. The increased numbers of bacteria (Table 1),

the disappearance of the bands related to cyanobacteria from the DGGE patterns (Fig. 1), and the increase in bacterial richness (Fig. 2) suggest a change in the bacterial community structure driven by the viral outbreak. This alleged viral control was also shown by the NMDS analysis of the DGGE patterns (Fig. 3), where notable changes in the banding patterns and thus in the community structure were observed during the lysis in both LSEs. This structuring capability of natural viruses was also shown by monitoring the removal of a specific bacterium that was introduced to a microcosm (25). Although the largest changes in the community structure occurred during the lysis of the cyanobacterial populations, small changes in the community structure before and after the lysis of cyanobacteria were apparent. These changes could have been caused by the increase in temperature at the start of the experiment or by the selectivity of the culture medium.

**Bacterial community structure.** We not only showed the removal of specific members of the microbial community, but we also detected their replacement by other species. Sequence analysis of the most dominant emerging bands showed that the majority of the newly appearing bacteria belonged to the *Cytophagales* (bands 1, 2, 4, and 7) and the actinomycetes (bands

TABLE 4. Sequence similarities of the excised eukaryotic bands that appear in Fig. 4<sup>a</sup>

Band		Closest relative		
No.	% Similarity	Species	Accession no.	Taxonomic description
1	89.5	<i>Entrophospora</i> sp.	Z14011	Fungi
2	98.2	<i>Cyclidium glaucoma</i>	Z22879	Ciliophora
3	84.6	<i>Gymnodinium beii</i>	U37406	Dinophyceae
4	82.5	<i>Paulinella chromatophora</i>	X81811	Euglyphina
5	93.1	<i>Scypha ciliata</i>	L10827	Porifera
6	99.4	<i>Brachionus plicatilis</i>	U49911	Rotifera
7	99.4	<i>Brachionus plicatilis</i>	U49911	Rotifera
8	91.4	<i>Cymindis punctigera</i>	AF002773	Arthropoda
9	97.5	<i>Brachionus plicatilis</i>	U49911	Rotifera

<sup>a</sup> Sequences were aligned to their closest relatives from the EMBL database. The similarity was calculated with gaps and ambiguities not taken into account.

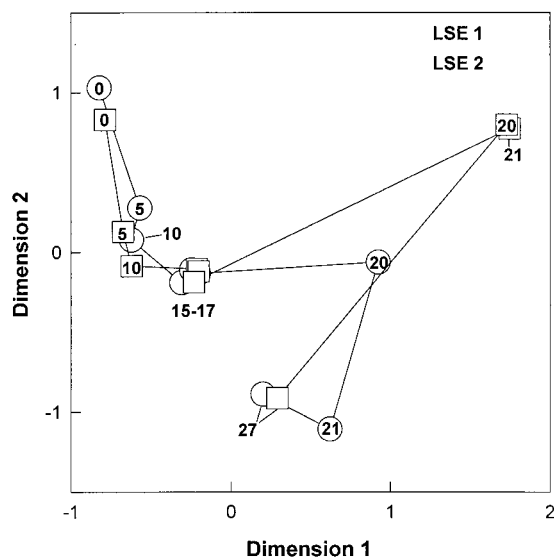


FIG. 5. NMDS map showing the changes in the structures of the eukaryotic communities during the lysis event. The numbers inside the symbols refer to the days of the experiment.

10, 11, and 12) (Table 3). Apparently, these bacteria can respond rapidly to the dissolved organic matter released due to the lysis. The *Cytophagales* are common soil and water bacteria (14) and are well known for their capability to degrade large complex carbohydrates (43). The actinomycetes are also found in freshwater habitats, where they may play an active role in the decomposition of chitin, cellulose, and proteins (26, 28, 29). One sequence (bands 5 and 6, which have identical positions in the gel) was related to the  $\beta$ -*Proteobacteria* and was 100% identical to that of an unknown bacterium isolated from the surface of a copper pipe used in a potable-water system (11). One sequence (bands 8, 9) was related to the  $\alpha$ -*Proteobacteria*. Both of these  $\alpha$ - and  $\beta$ -*Proteobacteria* are commonly found in freshwater environments (38).

Some bands that appeared at the same position in the DGGE patterns of LSEs 1 and 2 were excised from both patterns and sequenced to validate the identity of the sequences they exhibited. From bands 5 and 6, we did retrieve almost-identical sequences (98.2%). However, from bands 8 and 9 we retrieved different sequences. Additional sequencing of one more clone from each band revealed that of the four sequences obtained from bands 8 and 9, three sequences were nearly identical (99.4 to 100%) and one sequence (band 8a) was only 91.7% similar to the other sequences (bands 8b and 9). Apparently, these two sequence types were not resolved by DGGE.

**Eukaryotic community structure.** While the bacterial community showed almost-immediate changes after the lysis event, the eukaryotic community did not react until 5 days later. At that time, the pattern was completely dominated by a single band representing a rotifer-like sequence. After this apparent rotifer dominance, the community returned to a structure similar to that before the lysis. We found high numbers of rotifers in both LSEs, up to  $7 \times 10^4$  liter<sup>-1</sup>. While these numbers are commonly found in laboratory cultures, where densities can exceed  $10^6$  liter<sup>-1</sup> (55), the maximum natural abundance is at least four times lower (21). These rotifers feed on bacteria, phytoplankton, and protozoa (reference 3 and references therein), and the microbial loop after lysis apparently channeled the food for the rapid growth of these rotifer species.

Rotifers are considered to be an important link between the microbial loop and higher trophic levels (2, 40). The observed rotifer proliferation suggests that viral lysis can increase the carbon flow to the higher trophic levels, at least in freshwater systems like Lake Loosdrecht, where rotifers can rapidly reach high densities (21). Besides this rotifer-related band, we found one ciliate-related band and five bands that represented sequences with low similarities (<94%) to the limited number of known eukaryotic sequences in the EMBL sequence database. This low similarity may be partly caused by the direct sequencing of excised DGGE bands. We found that this method often yields ambiguous sequences.

**Conclusions.** The combination of a molecular profiling technique and an ordination method allowed the investigation of the impact of a viral lysis event on the structures of both the bacterial and the eukaryotic communities. Shortly after the cyanobacteria started to lyse, previously undetected bacterial sequence types were emerging in the DGGE profiles. The majority of these sequence types were related to bacterial species that are capable of degrading complex carbons. A few days later, a rotifer species profited from the lysis event and became dominant. Since rotifers are important links between the microbial loop and the classical food chain, this dominance after the viral outbreak could indicate the importance of viruses in channeling carbon through food webs.

The NMDS analysis of DGGE patterns seems to provide us with an interpretable, albeit qualitative, picture of the changes that occurred after the lysis of the cyanobacterial population. The NMDS analysis showed that the changes in the structures of both the bacterial and eukaryotic communities were remarkably similar in both LSEs (Fig. 3 and 5). This supports the notion that changes in community structure following changes in environmental conditions are not random and that these changing conditions act as governing forces of the microbial community structure.

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