Microbial Ecology

Effect of Nutrient Loading on Bacterioplankton Community Composition in Lake Mesocosms

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Received: 23 March 2005 / Accepted: 11 August 2005 / Online publication: 31 January 2006

Abstract

Changes in bacterioplankton community composition were followed in mesocosms set up in the littoral of Lake Vesijärvi, southern Finland, over two summers. Increasing nitrogen and phosphorus concentrations in the mesocosms represented different trophic states, from mesotrophic to hypertrophic. In 1998, the mesocosms were in a turbid state with a high biomass of phytoplankton, whereas in 1999, macrophytes proliferated and a clear-water state prevailed. The bacterial communities in the mesocosms also developed differently, as shown by denaturing gradient gel electrophoresis profiling of partial 16S rRNA gene fragments and by nonmetric multidimensional scaling analysis. In 1998, nutrient treatments affected the diversity and clustering of bacterial communities strongly, but in 1999, the bacterial communities were less diversified and not clearly affected by treatments. Canonical correspondence analysis indicated that bacterioplankton communities in the mesocosms were influenced by environmental physicochemical variables linked to the increasing level of eutrophication. Nitrogen concentration correlated directly with the bacterioplankton composition. In addition, the high nutrient levels had indirect effects through changes in the biomass and composition of phyto- and zooplankton. Sequencing analysis showed that the dominant bacterial divisions remained the same, but the dominant phylotypes changed during the 2-year period. The occurrence of *Verrucomicrobia* correlated with more eutrophic conditions, whereas the occurrence of *Actinobacteria* correlated with less eutrophic conditions.

Introduction

Eutrophication of an aquatic system is commonly caused by long-term nutrient discharge into the water as a result of human activities. Shallow lakes are especially at risk of becoming turbid, a state characterized by high phytoplankton biomass and poor water quality [34]. The natural and recreational value of the lake consequently deteriorates.

Bacteria play a key role in the microbial community responsible for recycling of the nutrients in the aquatic systems [4].

Numerous studies have been conducted on the regulation of bacterial communities by environmental factors at the biomass level. Recently, the development of molecular biology tools suitable for ecological studies has made it possible to follow changes in the composition of populations at the genetic level and the spatial and temporal effects on the populations as a consequence of environmental changes. DNA-based techniques, such as cloning and sequencing, fluorescent *in situ* hybridization, and denaturing gradient gel electrophoresis (DGGE), have particularly expanded our understanding of the diversity and identity of microbes in various habitats [1, 25, 43].

Changes in functioning of the bacterial community have been shown to be related to changes in its species composition [7, 18, 31]. Large, controlled mesocosm experiments in seawater have included studies on genotypic changes in bacterioplankton community composition in response to changes in organic substrate or inorganic nutrient concentrations [3, 27, 31, 33]. A few

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controlled studies have also been conducted in freshwater mesocosms [8, 22, 32, 48]. In addition, studies have been conducted to follow the development of the structure of aquatic microbial communities in response to changes in environmental conditions in eutrophic lakes [12, 20, 23, 41]. However, in freshwaters, the littoral zone has received little attention, although its habitats are more varied and the interactions are more complex than those in the pelagial zone. Thus, more experimental studies are needed to better understand the effects of increasing nutrient concentrations on the food web, including bacteria, in shallow lakes under threat of eutrophication.

We recently conducted a mesocosm study on the effects of nutrient enrichment and fish manipulation on phyto- and zooplankton and macrophytes in the littoral of mesotrophic Lake Vesijärvi in southern Finland [11]. The mesocosms were set up in two successive summers. In 1998, when macrophytic growth and cladoceran biomasses were low, chlorophyll *a* concentration increased linearly with phosphorus concentration, and mesocosms became turbid; only three control mesocosms without fish maintained a clear-water state. In 1999, by contrast, the nutrient additions were more moderate, and all the mesocosms maintained a clear-water state. Phytoplankton biomass remained low as a result of grazing by abundant cladocerans and reduced light penetration into water caused by macrophytes, especially freely floating lemnids (*Lemna trisulca* L.). Weather conditions in spring and early summer appeared to have been largely responsible for the differences in development of macrophytes and zooplankton in the 2 years.

In this article, we report on changes in bacterioplankton community composition (BCC) in the mesocosms. Mesocosms with different nutrient concentrations were selected to represent different levels of eutrophication, from mesotrophic to hypertrophic. To follow the changes in BCC, we used DGGE profiling of partial 16S rRNA gene fragments and nonmetric multidimensional scaling (NMDS). The correlations between the patterns obtained and environmental factors were investigated using canonical correspondence analysis (CCA). In addition, the phylogenetic affiliation of the major DNA bands was examined by DNA sequencing.

Materials and Methods

Description of Study Site and Experimental Design. Lake Vesijärvi in southern Finland, in its present state, is mesotrophic. Total phosphorus concentrations in the littoral zone outside our mesocosms varied between 5 and 50 $\mu g\ L^{-1}$, and total nitrogen (in 1999) varied between 600 and 1400 $\mu g\ L^{-1}$. Two factorial field mesocosm experiments were conducted starting on 4 June 1998 and 29 June 1999. The mesocosms were plastic cylinders of 1-m diameter that reached the lake bottom at 1-m depth. The nutrients, nitrogen as KNO_3 and phosphorus as KH_2PO_4, were added weekly. The concentrations of added nutrients were different in the two experimental years (Table 1). In 1998, the nutrient enrichment levels N2 and N3 were so high that they led to hypereutrophic conditions, which hardly ever occur in Finnish lakes, although possible in central and southern Europe. Therefore, in 1999, the nutrient additions were adjusted to represent mesotrophic to eutrophic conditions more relevant to the Finnish environment. Consequently, N1 in 1998 (100 $\mu g\ P\ L^{-1}$ and 1 mg $N\ L^{-1}$) was comparable with N3 in 1999 (90 $\mu g\ P\ L^{-1}$ and 0.9 mg $N\ L^{-1}$). In 1998, roach (*Rutilus rutilus* L.) and, in 1999, perch (*Perca fluviatilis* L.; both native species in the lake) were used in the experiments (Table 1). Additional lake water samples were taken just outside the mesocosms. The details of the experimental design have been published elsewhere [11, 38].

Sample Treatment. Water samples for the bacterial community analyses were taken from the mesocosms on the dates indicated in Table 1. Sampling always took place before nutrient additions. A 1-m-long tube was used to transfer 1 L of water into plastic bottles, which were then transported to the laboratory on ice.

For DNA extraction, 900 mL of sample water was concentrated by centrifugation at 4°C to approximately 500 μL. DNA was extracted using bead beating and CTAB (hexadecylmethylammonium bromide) treatment, purified with a Prep-A-Gene Purification Kit (Bio-Rad), and amplified with eubacterial 16S rRNA gene primers F-968-GC and R-1401, as in [10]. DCode™ Universal Mutation Detection System (Bio-Rad) was used for running the DGGE gels with 6% polyacrylamide and 35–50% formamide–urea gradient, as in [16]. The band intensities from the lake water samples were faint compared with the other samples, which was also reflected in the relatively low bacterial DAPI counts (results not shown). We therefore verified the lake banding patterns by running an additional gel, for which we obtained more DNA by combining several replicate polymerase chain reactions (PCRs).

Strong and well-defined bands were cut from the DGGE gels, sequenced, and compared with databank entries, as in [10].

Statistical Analyses. Denaturing gradient gel electrophoresis images were transferred to GelCompar software (Applied Maths, version 4.1) for alignment of the electrophoresis patterns. The alignment was corrected by visual inspection of the gels. An optical density profile was measured for each DGGE lane (sample), and the band positions and relative (%) intensity of each band were calculated against the whole lane (100%). The data matrix obtained based on the relative DNA band intensities was used for statistical analyses.

Table 1. Sampling dates, corresponding experimental week, and a number of samples from the lake water and mesocosms analyzed using denaturing gradient gel electrophoresis (DGGE)

				Phosphorus and nitrogen enrichment levels in the mesocosms													
				N0		*N1*		*N2*		*N3*		*N1*		*N2*		*N3*	
	Sampling date	*Exp. week*	*Lake water*	*0*	*0*[a]	*100*	*1*	*500*	*5*	*1000*	*10*	*30*	*0.3*	*60*	*0.6*	*90*	*0.9*
Fish[b]				−	+	−	+	−	+	−	+	−	+	−	+	−	+
1998	6 July	4	2	2[c]		1	2	2	2								
	20 July	6	2	2	1	2	2	2	2								
	3 August	8	2	2	1	2	2	2	2								
	10 August	9	1		1	1	2	2	2	2	2						
1999	29 June	0		2	2							2	2	2	2	2	2
	6 July	1	1	1	1							1	2			1	1
	13 July	2	1	2	2							2	2	2	2	2	2
	27 July	4	1	2	2							2	2	2	2	2	2
	10 August	6	1	2	2							2	2	2	2	2	2

[a] Enrichment levels of phosphorus (μg L^{-1}) and nitrogen (mg L^{-1}).
[b] Fish: −, absent; +, present at 20 g FW m^{-2}.
[c] When two samples were taken from mesocosms, they were from different mesocosms receiving the same treatment.

NMDS was used to cluster the samples. NMDS analyses were performed with the Community Analysis Package software (version 2.0, Pisces Conservation Ltd., Lymington, UK), which calculated the Sorensen similarity coefficients and visualized the similarities between the samples in two dimensions. CCA, a direct gradient analysis method in the CANOCO 4.5 software package [40], was used to compare the DGGE band matrices with the environmental variables (physicochemical variables and phyto- and zooplankton data). Both DGGE data and environmental variables (except pH, alkalinity, temperature, and total suspended solids) were $\log(x + 1)$-transformed. Forward selection was used to rank the environmental variables in importance for determining the species data, and the Monte Carlo permutation (499 permutations) test was applied to judge which variables contributed significantly to the statistical model. The environmental (explanatory) variables used were fish density, nitrogen concentration measured as NO_3, NH_4, NO_2 (in 1998), and total nitrogen (in 1999), total phosphorus concentration, soluble reactive phosphorus (in 1999), transparency as determined by Secchi depth, pH, alkalinity (in 1998), water temperature, oxygen concentration and saturation, total suspended solids (in 1998), chlorophyll *a*, biomasses of the phytoplankton groups [(in μg L^{-1}): cyanobacteria, diatoms, dinoflagellates, chloro-, chryso-, conjugato-, crypto-, eugleno-, and tribophytes, phytoflagellates], and biomasses of the zooplankton groups [(in μg C L^{-1}): rotifers, large euplanktonic grazers (>0.5 mm; e.g., *Daphnia*, *Eudiaptomus*), small euplanktonic grazers (<0.5 mm; e.g., rotifers, *Bosmina*, *Ceriodaphnia*, nauplii), large plant-associated Cladocera, small plant-associated Cladocera, *Polyphemus*, calanoid copepods (*Eudiaptomus*), cyclopoid copepods, nauplii, large grazers, small grazers, crustacean grazers, grazers, raptorials, and all crustaceans]. Measurement of the environmental variables has been described previously [11].

Results

Analysis of DGGE Fingerprint Patterns. Denaturing gradient gel electrophoresis fingerprint patterns were obtained for 50 samples from 1998 and for 75 samples from 1999 (Table 1). In general, in 1998, the bands were dispersed across the entire gel gradient, whereas in 1999, they were more concentrated in the middle (Fig. 1). The

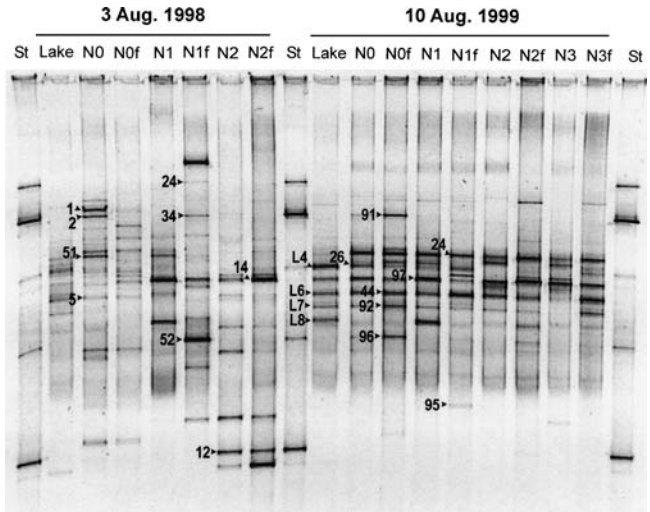

Figure 1. Example of denaturing gradient gel electrophoresis (DGGE) fingerprint patterns obtained from the water samples in 1998 and 1999. St: standard; Lake: lake water; N0, N1, N2, and N3: different nutrient levels (Table 1); f: fish. For the numbered bands, a matching sequence is shown in Table 3.

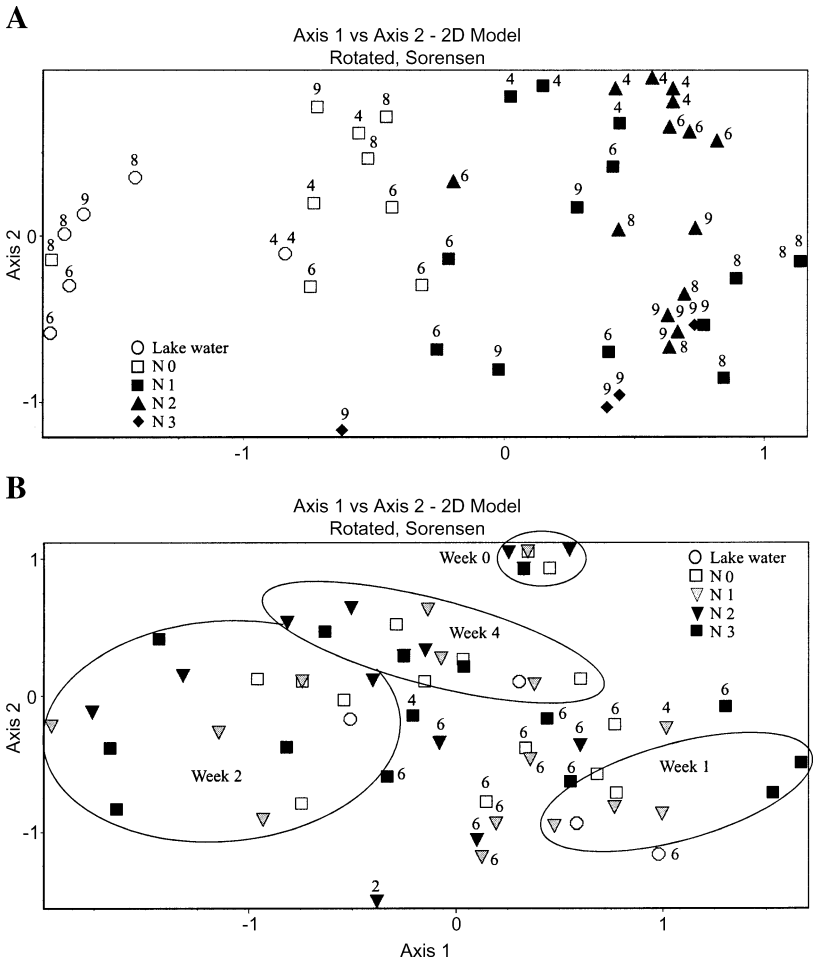

Figure 2. Clustering of the DGGE fingerprint patterns by nonmetric multidimensional scaling for (A) the 1998 samples and (B) the 1999 samples. Nutrient levels as in Table 1. Numbers next to the symbols show the sampling week.

fingerprints of the lake samples remained relatively constant over the sampling period of both years. The same main bands were present, although their intensities varied and a few unique bands were seen (Fig. 1).

NMDS analysis was used to display the relations of the fingerprint patterns in two dimensions (Fig. 2). In 1998, the succession of bacterial communities was affected by the nutrient treatments, even at the lowest nutrient addition level N1, whereas in 1999, the banding patterns in all the treatments remained closer to that of the lake water. In 1998 (Fig. 2A), the samples clustered according to the nutrient treatment; thus, the lake samples and control mesocosms clustered on the left side of the plot, and the general shift of the patterns to the right took place with the increasing nutrient addition levels. In addition, in the mesocosms with high nutrient addition levels, the samples from the early weeks of the experiment were found on the top half, and the samples from the later weeks were found on the bottom half of the plot. In 1998, the so-called bottle effect was detected because the fingerprint patterns of the control mesocosms consistently differed from those of the lake samples. However, the patterns were closer to the ones from the lake water than to the treated mesocosm patterns. The final stress of the first dimension was 0.3606 and of the second dimension 0.245.

In 1999, the first sampling was performed before the first nutrient addition and at the time the patterns

Table 2. Summary of CCA results on bacterial community composition constrained to the significant ($p < 0.05$) environmental variables

	1998		*1999*	
No. of samples	50		75	
No. of species (DNA bands)	52		35	
No. of significant environmental variables	8		10	
Eigenvalues (for the second and fourth axes)	0.415	0.277	0.305	0.151
Species–environment correlations	0.908	0.847	0.919	0.903
Cumulative percentage variance:				
Species data	14.6	23.0	17.0	25.9
Species–environment relation	45.9	72.2	46.2	70.1
Sum of all eigenvalues	7.224		3.923	
Sum of all canonical eigenvalues	2.304		1.447	

from different mesocosms were very similar to each other (Fig. 2B). Samples taken during the experiment clustered according to the sampling week, indicating succession of the populations over time, regardless of the experimental treatments. In the 1999 analysis, the final stress of the first dimension was 0.3863 and of the second dimension 0.2112.

CCA was used to investigate the correlations between DGGE banding patterns and environmental variables, including physicochemical water properties and different groups of phyto- and zooplankton. The CCA plot of samples, species, and significant environmental variables based on the first four axes explained 23.0% in 1998 and 25.9% in 1999 of the variance in species data (Table 2). In 1998, the environmental factors contributing significantly to the statistical model were pH, concentrations of nitrate, chlorophyll *a* and total suspended solids, and biomasses of cryptophytes, cyanobacteria, small (<0.5 mm) euplanktonic grazers, and cyclopoid copepod nauplii (Fig. 3A). In clustering of the 1998 samples, nutrient treatment had the most pronounced effect; samples from the lake and control mesocosms were placed on the left side of the plot, and samples from the nutrient-enriched mesocosms were placed on the right side together with the vectors indicating increasingly eutrophic conditions. Clustering of the latter samples also changed over time. In 1999, the environmental factors contributing significantly to the model were concentrations of total nitrogen, nitrate, and oxygen, oxygen saturation, temperature, and biomasses of crypto- and chlorophytes, diatoms, crustacean grazers, and zooplankton grazers (Fig. 3C). In 1999, the samples clustered according to the sampling date, whereas the nutrient treatment did not seem to have an effect.

Analysis of Partial 16S rRNA Gene Sequences. Major bands were excised from the DGGE gels, re-amplified, and sequenced. Sequences were obtained for 15 of the 52 bands used in CCA analysis in 1998 and for 14 of the 35 bands in 1999. The sequenced bands

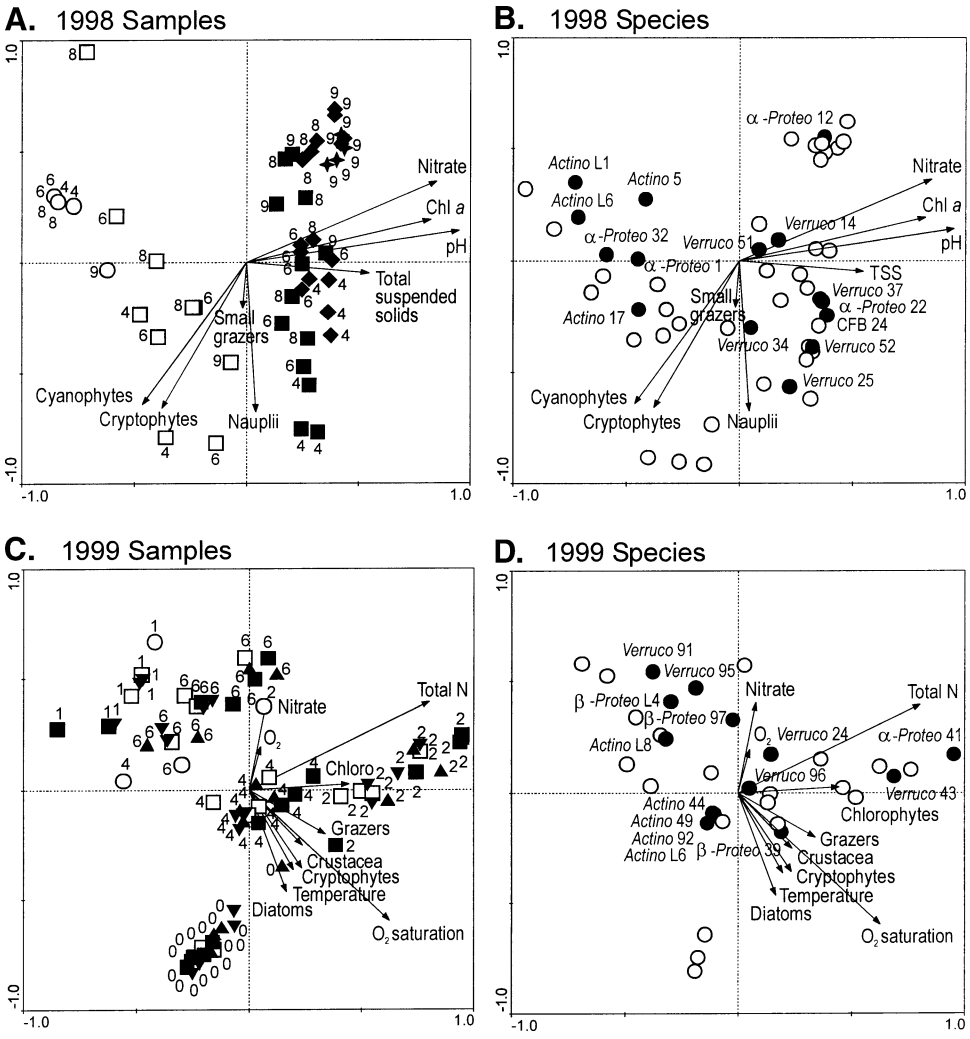

Figure 3. Canonical correspondence analysis ordination plots of the samples, species (DGGE bands), and environmental variables. Symbols (A, C): open circle, lake water; open square, control mesocosm N0; black square, nutrient levels N1 (1998) and N3 (1999); black diamond, N2 (1998); black star, N3 (1998); down-triangle, N1 (1999); up-triangle, N2 (1999). Numbers next to the symbols show the sampling week. Symbols (B, D): black circle, sequenced band; open circle, band not sequenced. Numbers refer to the matching sequences in Table 3.

comprised 59 and 84% of the total band intensity in 1998 and 1999, respectively. Some strong bands gave mixed sequences because of DNA from several organisms present in close proximity; these were not analyzed further. Altogether, 22 different unambiguous sequences were obtained (Table 3). These sequences represented three divisions: *Verrucomicrobia*, *Actinobacteria*, and *Proteobacteria*. In addition, one sequence from the *Cytophaga–Flavobacterium–Bacteroides* (CFB) group was obtained.

In 1998, band VE14-98 represented a very common verrucomicrobial phylotype. It had a novel sequence and possessed only 95% similarity to the closest reported relative. At week 4, it was present in lake water and mesocosms, but thereafter only in mesocosms. In several cases, it represented over half of the total band intensity in nutrient-enriched mesocosms. In 1999, VE24-99 was the predominant verrucomicrobial phylotype, present in both lake water and mesocosms. In 1998, *Actinobacteria* were present mainly in lake water and in control mesocosms; one actinobacterial band could represent as much as one third of the total band intensity of the lane. In 1999, actinobacterial VE44-99 was common in all but the highest nutrient-enriched mesocosms. Thus, for both

Table 3. Sequenced DGGE bands from the water samples from years 1998 and 1999 and their closest relatives

Taxonomic description	*Sequence designation*[a]	*Accession number*	*Similarity (%)*	*Closest relative*[b] *in databank (accession no.) and source*
Verrucomicrobia	VE14-98	AJ507498	95	AH9 (AJ629851) Finland: Lake Ahvenlammi
Verrucomicrobia	VE25-98	AJ507501	99	MAH1 (AJ629850) Finland: Lake Ahvenlammi
Verrucomicrobia	VE34-98, VE2-98, VE91-99	AJ507504	98	*Prosthecobacter fusiformis* (U60015)
Verrucomicrobia	VE37-98	AJ507505	100	Jo146-01 (AJ620837) Finland: Lake Joutikas
Verrucomicrobia	VE52-98, VE96-99	AJ507511	99	HTBB2 (AF418948) USA: Horsetooth Reservoir
Verrucomicrobia	VE51-98, VE24-99	AJ507510	100	NE55 (AJ575738) Germany: Lake Grosse Fuchskuhle
			100	CR99-2-04 (AF429190) China: Changjiang River
Verrucomicrobia	VE43-99	AJ507507	99	NE55 (AJ575738) Germany: Lake Grosse Fuchskuhle
			99	CR99-2-04 (AF429190) China: Changjiang River
Verrucomicrobia	VE95-99	AJ507513	100	S4.13 (AY752115) France: Sep Reservoir
Actinobacteria	VE5-98, VEL6-99	AJ507509	100	CR99-35-08 (AF428966) China: Changjiang River
			100	S9F-17 (AB154306) Japan: Lake Kasumigaura
Actinobacteria	VE44-99, VE17-98	AJ507508	100	CR99-2-40 (AF429226) China: Changjiang River
			100	S9F-52 (AB154310) Japan: Lake Kasumigaura
Actinobacteria	VEL1-98, VE49-99	AJ507515	100	R6 (AJ575502) Czech Republic: Rimov Reservoir
			100	HT2E3 (AF418967) USA: Horsetooth Reservoir
			100	CRP99-50 (AF428704) China: Lake Poyang
			100	S9F-07 (AB154305) Japan: Lake Kasumigaura
Actinobacteria	VE92-99, VEL6-98, VEL7-99	AJ507512	99	MAH5 (AJ629849) Finland: Lake Ahvenlammi
			99	R5 (AJ575501) Czech Republic: Rimov Reservoir
			99	CR99-24-76 (AF429186) China: Changjiang River
			99	S9F-17 (AB154306) Japan: Lake Kasumigaura
Actinobacteria	VEL8-99	AJ507517	100	R1 (AJ575497) Czech Republic: Rimov Reservoir
			100	CR99-7-45 (AF429079) China: Changjiang River
α-*Proteobacteria*	VE1-98	AJ507496	94	Clone 5 (AJ579581) French Guiana: rhizosphere
α-*Proteobacteria*	VE12-98	AJ507497	99	Isolate MB15 (AY328845) USA: drinking water
α-*Proteobacteria*	VE22-98	AJ507499	97	LiUU-9-283 (AY509406) Sweden: Lake Limmaren
			97	CRD98-24-29 (AF428835) China: Changjiang River
			97	*Rhodobacter* sp. JipO3 (AB122032) rotten rice straw
α-*Proteobacteria*	VE32-98	AJ507503	97	CRD98-35-68 (AF428950) China: Changjiang River
			97	Isolate R-23041 (AJ786815) nitrifying inoculum
			97	Isolate SW2 (X78717)
α-*Proteobacteria*	VE41-99	AJ507506	99	LiUU-9-283 (AY509406) Sweden: Lake Limmaren
			99	CRD99-18 (AF428596) China: Lake Dongting
β-*Proteobacteria*	VE26-99, VE39-99	AJ507502	100	CR99-24-32 (AF429142) China: Changjiang River
β-*Proteobacteria*	VE97-99	AJ507514	100	CR99-2-71 (AF429257) China: Changjiang River
β-*Proteobacteria*	VEL4-99	AJ507516	99	P38.4 (AY752086) France: Lake Pavin
			99	PRD01b009B (AF289169) USA: Parker River
			99	CR99-7-02 (AF429036) China: Changjiang River
			99	*Variovorax* 444D (AY571832) Antarctica: soil
CFB	VE24-98	AJ507500	93	CR99-35-71 (AF429029) China: Changjiang River

[a] VE: from the mesocosms in Lake Vesijärvi; VEL, from lake water outside the mesocosms.
[b] Uncultured bacterium unless otherwise stated.

years, the strong actinobacterial bands were limited to mesocosms having less than 100 μg P L^{-1} and 1 mg N L^{-1}. In addition to actinobacterial sequences, only one verrucomicrobial sequence and one β-proteobacterial sequence were obtained from the lake water. In the mesocosms, the occurrence of sequenced *Proteobacteria* was rather sporadic; different phylotypes could prevail in a couple of mesocosms each week. The CFB sequence was detected only during week 4 in 1998.

CCA analysis showed the bands detected in DGGE gels in relation to the environmental variables (Figs. 3B, D). *Actinobacteria* were correlated with less eutrophic conditions in the CCA, whereas *Verrucomicrobia* and the only representative of the CFB group were correlated with more eutrophic conditions.

Discussion

Changes in Bacterioplankton Community Composition. In 1998, nutrient treatments affected the diversity and composition of bacterial communities strongly. In addition, probably another unknown factor (or factors) changed over time and affected the bacterial communities. CCA analysis indicated that BCC of nutrient-enriched mesocosms was influenced by the environmental variables related to an increased level of eutrophication (high nutrient, chlorophyll *a*, and total suspended solids concentrations and pH). Nutrient enrichment clearly increased the biomass of phytoplankton [11]; however, only the concentration of nitrogen (measured as nitrate in 1998) correlated directly with BCC. In Lake Vesijärvi, shortage of inorganic nitrogen has been reported to limit the growth of algae [26], but in the quantities available in our mesocosms (trophic levels from mesotrophic to eutrophic), we did not expect that nitrogen or phosphorus limitation would have affected BCC directly. High biomass of phytoplankton increased photosynthesis rate, which led to elevated pH values. In fact, pH in the high-nutrient mesocosms rose so much (pH 11) that all fish died. A correlation between pH and BCC has also been reported elsewhere [20, 37, 47]. pH may merely reflect changes in other environmental factors, such as phytoplankton, but an increase in pH may also influence BCC directly or indirectly by, for example, changing the solubility of phosphorus [17].

In 1999, the bacterial communities were less diversified and resembled that of the lake water community. However, BCC changed to some extent over time. Seasonal succession of freshwater bacterioplankton has been attributed to such factors as light, temperature, wind-induced currents, and phytoplankton blooms [5, 12, 51]. In our CCA analysis, temperature and temperature-dependent oxygen saturation emerged as significant physical environmental factors. Concentration of nitrogen (measured as total N and nitrate in 1999) again correlated significantly with BCC, but chlorophyll *a* did not. In 1999, the nutrient levels were generally lower than in 1998, but still at the highest nutrient level of 1999 (90 μg P L^{-1} and 0.9 mg N L^{-1}), abundant nutrients were available in the nutrient-enriched mesocosms. However, phytoplankton biomass remained low in 1999 because of grazing by abundant cladocerans. Macrophytes, which dominated the mesocosms in 1999, could be expected to have a direct effect on the attached bacterial communities rather than on planktonic bacterial communities [46].

Certain phytoplankton groups correlated significantly with BCC in both years. In 1998, cyanobacteria and cryptophytes were abundant in the control mesocosms and lake water. Nutrient-enriched (eutrophic to hypertrophic) mesocosms had a high chlorophyll *a* concentration, mainly because of the great biomass of the chlorophyte *Scenedesmus* spp. In 1999, when the nutrient concentrations were lower and closer to the actual ones in Northern European lakes, the significant phytoplankton classes were cryptophytes, diatoms, and chlorophytes. These results are consistent with those obtained from mesotrophic lakes in Sweden [21]. Phytoplankton is a major source of autochthonous organic matter in lakes, and algal exudates are highly bioavailable to bacteria compared with other sources of dissolved organic matter (DOM) [36, 45]. The species composition, physiological state, and biomass of phytoplankton seem to regulate the photosynthetic extracellular release, which, in turn, may influence the composition of bacterial communities [24, 28, 30, 31, 42]. When we examined the role of the phytoplankton species more closely, different species from several phytoplankton classes showed significant correlations with BCC (results not shown). However, controlled experiments are needed to verify whether the presence or absence of these species can, in fact, lead to different BCC.

Top-down control, i.e., grazing and viral infections, also plays an important role in the control of microbial communities. In our mesocosms, we did not examine protists, the bacterivores such as heterotrophic nanoflagellates and ciliates, or the effect of viruses. However, biomasses of micro- and mesozooplankton were determined and reported by Hietala *et al.* [11]. In 1998, small grazers, predominantly rotifers and naupliar stages of cyclopoid copepods, correlated significantly with BCC. The rotifer community was dominated by microphagous species such as *Keratella cochlearis* and small *Trichocerca*, which readily feed on bacteria-sized particles [29]. In their mesocosm tracer studies on carbon cycle, Lyche *et al.* [23] showed that zooplankton community comprised mainly of cyclopoid copepods was weakly coupled to microbial loop. Thus, the correlation between BCC and nauplii might have been a result of other interactions in the complex food web or might have been merely

coincidental. In 1999, the general groups of grazers and crustaceans, which predominantly consisted of filter-feeding cladocerans, correlated significantly with BCC. The predominant zooplankton thus seemed to influence BCC, either directly or by selective feeding on protists, which, in turn, influenced BCC, as also reported earlier [12, 14, 15, 19, 20, 33, 48]. In addition, grazers act as producers of DOM through "sloppy feeding", feces, and other excreta. Grazers release nutrients in organic rather than in inorganic forms, thus supporting heterotrophic rather than autotrophic processes in ecosystems [2]. Also, fish may efficiently return organic and inorganic substrates into the water column through excretory processes and by mixing of sediment [13] and thus enhance bacterial growth. Nevertheless, fish did not affect the clustering of BCC in our study.

In relating the changes in BCC patterns to environmental variables in CCA, we chose to use band intensity data because we felt that they better depict the changes taking place in the bacterial community than absence/presence data, as also stated by Muylaert *et al.* [24] and Yannarell and Triplett [47]. No doubt PCR amplification can severely distort the relative proportions of different genotypes, but because we compared samples originating from the same environment at different time points, the quantitative results can be assumed to reflect the proportional changes taking place in BCC. In addition, caution is needed when considering the significance of the environmental factors. Transformation of data can have an impact on the results, as shown by Yannarell and Triplett [47], and the significance of a single environmental factor also depends on the other environmental variables included in the run. It is also possible that some of our unsequenced bands may have originated from cyanobacteria or chloroplasts, thus increasing the correlation we detected between BCC and the phytoplankton groups.

Sequence Diversity. The bacterial groups we detected by sequence analysis are among the universal groups found in freshwater environments [49]. Most of our sequences were closely related to previously reported sequences from uncultured freshwater bacteria. Close relatives were typically found from distant geographic locations.

The common occurrence of *Verrucomicrobia* in our mesocosms suggests that *Verrucomicrobia* are able to take advantage of a nutrient-rich environment, or that the phylotypes represented by our sequences had some features that made them competitive in a confined environment. Many *Verrucomicrobia* are prosthecate [50], and this could offer them an advantage in nutrient uptake or make them more resistant to grazing. Our studies on BCC in other Finnish lakes have also indicated that *Verrucomicrobia* are relatively more prevalent in eutrophic lakes than in oligo- or mesotrophic lakes [10, 16].

Another group of bacteria commonly present in our samples was *Actinobacteria*. Four of our five sequences belonged to actinobacterial clade acI and one (VEL8-99) to clade acII, as defined by Warnecke *et al.* [44]. Both of these clades harbor 16S rRNA sequence types from freshwaters and estuaries with varying hydrological and limnological characteristics and from distant geographic locations. Besides mesotrophic Lake Vesijärvi, we have found closely related *Actinobacteria* in other Finnish lakes of different nutrient and humic content as well as in cyanobacterial blooms [16]. In the CCA analysis, *Actinobacteria* clustered far from the vectors indicating a high level of eutrophication. This supports our earlier notion that although *Actinobacteria* are permanent abundant members of bacterioplankton, they do not respond strongly to new nutrient resources [10].

Proteobacteria and the members of the CFB group have, in many studies, been found to be the predominant freshwater bacteria (e.g., [6, 9, 35, 49]). In our mesocosms, their occurrence was rather sporadic. However, because PCR amplification can distort the results [39], and we did not obtain sequences from every strong band in the gels, we cannot draw firm conclusions about the most common bacteria in the mesocosms.

In conclusion, with our mesocosm experiment, we have shown that when a shallow lake shifts from mesotrophic to eutrophic or hypertrophic, and clear-water state shifts to turbid state, BCC also changes considerably. High nutrient levels, especially nitrogen, appeared to induce a shift in BCC. In addition, nutrient loading had indirect effects on BCC through changes in the biomass and composition of phyto- and zooplankton. The 2-year experiment also showed that the results of environmental studies can be greatly influenced by natural variation in weather conditions in different years. Nevertheless, similar types of environmental variables did correlate with BCC for both years. The dominant bacterial divisions we detected remained the same over this period, although the dominant phylotypes were different.

The occurrence of *Verrucomicrobia* correlated with more eutrophic conditions and *Actinobacteria* with less eutrophic conditions.

Acknowledgments

We thank Totte Niittylä and Jaana Vaitomaa for technical assistance. Financial support for the study was received from the Academy of Finland (grants 395901 and 201576 to K.S. and grant 48827 to K.H.) and from the Maj and Tor Nessling Foundation to K.H. The mesocosm experiment was funded by the European Commission (grant ENV4-CT97-0420 to T.K.).

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