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Weak coupling between community composition and functioning of aquatic bacteria

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Abstract

We performed a batch culture experiment with a factorial design in which sterile water from four lakes and bacterial assemblages (size-fractionated lake water) from the same lakes were set up in all possible combinations. The functional performance (biomass yield, respiration, growth rates, and growth efficiency) of bacterial communities growing in the cultures depended primarily on the type of the medium and to a much lesser extent on the origin of the bacterial assemblage. Functional changes were only partly paralleled by changes in community composition, as indicated by terminal restriction fragment length polymorphism analysis. Similar bacterial communities developed in different cultures as a result of receiving either the same medium or the same inoculum, indicating that bacterial communities are comprised of populations of generalists that can grow under most conditions as well as populations with the life strategy of specialists. However, bacteria originating from a slightly acidic polyhumic lake failed to grow, grew unsteadily, or exhibited an extended lag phase when exposed to media originating from other lakes, indicating that the bacterial community in the polyhumic lake was not able to adapt rapidly to changes in environmental conditions.

Microorganisms are extremely abundant, proliferate rapidly, and disperse easily (Pedrós-Alió 1993; Whitman et al. 1998). Hence, the distribution of bacteria and other microbes may be largely independent of geographic barriers (Finlay 2002), i.e., the community composition in a given habitat should be determined by local environmental conditions, rather than by restricted dispersal. Accordingly, early investigators considered that "everything is everywhere, and the environment selects" (Baas-Becking 1934).

Several studies corroborate the ubiquitous distribution of microorganisms. For example, Finlay and Clarke (1999) found that a small sediment sample from an English pond contained remnants of as much as 80% of all globally identified species within the diverse eukaryotic flagellate genus *Paraphysomonas.* Fenchel et al. (1997) found that a small sediment sample contained an appreciable fraction of the globally occurring species of free-living ciliates. Most of these species were not detectable in the original sample, but could be triggered to grow by a variety of enrichment techniques, such as heating and substrate additions. Hence, although not actively growing, many species were present as a "seed bank," or cryptic diversity, because of the ubiquitous dispersal of microorganisms.

Studies have also shown that many aquatic prokaryotes have a cosmopolitan distribution (Zwart et al. 2002; Hahn 2003). On the other hand, an increasing number of studies on prokaryotes suggests that at least some organisms are endemic to certain regions (Papke et al. 2003; Whitaker et al. 2003). The degree of endemicity exhibited by microorganisms seems to be a matter of genetic resolution, since it has been shown that cosmopolitan distribution occurs on the genus level, whereas endemicity can be found on the subspecies level (Cho and Tiedje 2000).

Ecosystem functioning often depends on species composition, i.e., on exactly which species are present in a community (Mikola and Setälä 1998). In aquatic systems, for example, the presence of keystone species such as *Daphnia* has a strong impact on important ecosystem functions such as nutrient recycling and primary production (Mazumder 1994; Urabe et al. 2002). Recent studies on bacteria showed that changes in ecosystem functioning can be coupled to the genetic structure of bacterial communities (e.g., Findlay et al. 2003), suggesting that the magnitude and efficiency of ecosystem processes depends on bacterial community composition. However, it has also been shown that bacterial communities are functionally redundant, i.e., differently composed communities can carry through similar functions (Fernandéz et al. 2000).

Hence, there are conflicting results regarding the importance of community composition for the functional performance of bacterial communities, as well as contrasting results regarding the ubiquity of heterotrophic bacterial species. To integrate these two aspects of diversity and functioning of heterotrophic bacterial communities, we performed an experiment in which water from four different lakes served as medium for the growth of bacterial communities inoculated from the same four lakes in all possible combinations. We tested whether there were significant effects of the source of the medium or the origin of the inoculum on various functional properties (biomass yield, growth rate, respiration, and growth efficiency) and community composition (terminal restriction fragment length polymorphism [t-RFLP] analysis). We hypothesize that because of the high local diversity of bacterial communities, similar bacterial communities should develop in cultures in-

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Table 1. Experimental setup and assignment of treatment identifications. For treatment assignment, the first digit refers to the medium; the second one refers to the inoculum. The same numbers are used for the lakes.

Medium		Inoculum			
		Långsjön	Lötsjön	Ekoln	Tvigölingen
		1	2	3	4
Långsjön	1	1.1	1.2	1.3	1.4
Lötsjön	2	2.1	2.2	2.3	2.4
Ekoln	3	3.1	3.2	3.3	3.4
Tvigölingen	4	4.1	4.2	4.3	4.4

oculated with different bacterial starting communities, but where all other conditions are the same. This in turn should translate to functional similarity of cultures where conditions are similar, independently of the origin of the inoculum.

Material and methods

Sampling and experimental setup—We sampled four lakes differing in nutrient and dissolved organic carbon (DOC) concentration. From each of the lake waters a medium (0.2 μm filtered lake water) and an inoculum were prepared. Subsequently a two-factorial batch culture experiment was set up in all combinations, i.e., each medium was inoculated with each of the four inocula (Table 1). Each treatment consisted of four replicate bottles.

The lakes are located close to the city of Uppsala in mid-Sweden, and include an oligotrophic clear-water lake with high groundwater input (Långsjön; 60°03′N, 17°35′E), a eutrophic clear-water lake (Lötsjön; 59°52′N, 17°57′E), a eutrophic moderately humic lake (Ekoln; 59°45′N, 17°36′E), and an oligotrophic polyhumic forest lake (Tvigölingen; 60°05′N, 17°24′E). Some characteristics of the lakes are summarized in Table 2.

Water from Långsjön and Tvigölingen was collected on 11 October 2002, whereas Lötsjön and Ekoln were sampled on 14 October 2002. Surface water (1 m depth) was pumped through a 200-μm plankton net into acid-washed 25-liter polyethene bottles. Conductivity, alkalinity, and pH were measured within a couple of hours according to standard procedures. The water was stored in the dark at +4°C until further processing.

Water for the preparation of the medium was sequentially filtered through a polypropylene filter cartridge with a pore size of 5 μm (A-filter), a 142-mm AE glasfiber filter (Pall cooperation, precombusted for 8 h at 450°C), and finally through a 0.2-μm Mini Filter polysulfone capsule (Gelman Sciences). All filter units were excessively rinsed with Milli-Q water before use, and care was taken to avoid high pressure during the filtration process to prevent bursting of cells and unintended release of dissolved organic matter (DOM). Nitrogen and phosphorus were added as NH_4Cl and Na_2HPO_4, respectively, to yield final concentrations of 70 μmol L^{-1} N and 3.2 μmol L^{-1} P. Assuming an average bacterial C : N : P ratio of 45 : 7.4 : 1 (Vrede et al. 2002) it is therefore unlikely that N or P limitation occurred even in cultures where the highest biomass yields were obtained. Hence, we made sure that bacterial growth in all cultures was likely to be C limited. Since this experiment depended on a total absence of viable bacteria in the medium before inoculation, the 0.2-μm filtrate was autoclaved for 1 h, cooled, and stored at 20°C for 3–4 d and autoclaved again for 1 h. By this procedure we made sure that even spores that survived and were activated during the first autoclaving were killed. Alkalinity and pH in all four media after autoclaving were measured. Concentration of DOC in 0.2-μm-filtered original lake water samples was measured on a Shimadzu TOC analyzer after acidification as described previously (Langenheder et al. 2003). To characterize the DOC of the four different lakes absorbance of 0.2-μm-filtered water samples was measured over a wavelength spectrum of 200–500 nm using a Perkin-Elmer Lambda 40 spectrophotometer.

Before the start of the experiment sterile serum glass bottles (1.2 liters), sealed with teflon-coated butyl rubber stop-

Table 2. Characteristics of the studied lakes.

Lake	Temperature (°C)	DOC (mg L^{-1})	pH	Alkalinity (mekv L^{-1})	Tot-P (μg L^{-1})	Bacterial abundance (10^6 ml^{-1})
1	8	6.4	8.30	2.94	15 ± 4* (7.5–20)	1.72
2	7	9.7	8.01	1.63	30 ± 11* (15–53)	1.97
3	5	13.3	7.76	2.24	41 ± 8† (30–50)	1.69
4	8	40.9	6.82	0.48	7.5–15‡	3.77

* Mean value ± SD (range of minimum of maximum concentrations) obtained at different sampling dates between 1997 and 2003 ($N_{lake\ 1}$ = 9; $N_{lake\ 2}$ = 13). Measurements were done during the Limnology MN 1 course, Dept. of Limnology, Evolutionary Biology Centre, Uppsala University.

† Mean value ± SD (range of minimum of maximum concentrations) during 2002 (Weyhenmeyer 2003).

‡ Brunberg and Blomqvist (1997).

pers, were filled with sterilized water without headspace. Each bottle had an inlet reaching the bottom of the bottle, and an outlet from the top, that consisted of sterile Teflon tubing connected to sterile three-way valves.

The inoculum was prepared by filtering the lake water through GF/F filters (Whatman, precombusted for 8 h at 450°C). Ten milliliters of the filtrate was injected into each bottle, and the resulting batch cultures were incubated at 20°C in the dark. To retrieve samples, 5 to 10 ml of autoclaved and additionally filter-sterilized medium was injected into the inlet and samples withdrawn simultaneously from the outlet, to avoid air bubbles in the system. This procedure resulted in a dilution at each sampling occasion of the medium by at most 0.83%. Controls with only sterile medium but no inoculum were prepared (four for each medium) and samples were withdrawn at the beginning and end as well as at one intermediate time point.

All glass- and plasticware coming into contact with the samples were soaked for several hours in 1 mol L^{-1} HCl and rinsed with excessive amounts of Milli-Q water afterwards.

Oxygen consumption and DOC—The net change of dissolved oxygen concentration in the cultures was measured according to the spectrophotometric modification of the Winkler titrimetric method (Roland et al. 1999) in 30-ml Winkler flasks. The absorbance at 450 nm was measured and transferred into dissolved oxygen concentration using a calibration curve derived from a Winkler titration (Mille-Lindblom and Tranvik 2003). Bacterial respiration (BR) was defined as O_2 utilization in the cultures and transferred to carbon units assuming a respiratory quotient of 1 (del Giorgio and Cole 1998).

Bacterial abundance, biomass, growth, and growth efficiency—Subsamples of 5–20 ml were preserved with filter-sterilized formaldehyde at a final concentration of 2% (v/v). For bacterial abundance measurements samples of 500 μl were then stained with Syto 13 (Molecular probes) and analyzed flow cytometrically using a FACScan (Becton Dickinson) with minor modifications of the original protocol (del Giorgio et al. 1996). Samples from the end of the experiment were counted using a Nikon Eclipse E600 epifluorescence microscope after staining with Syto 13 (Mille-Lindblom and Tranvik 2003). The flow cytometric counts were slightly higher than the microscopic counts (linear regression, slope $= 1.18$, $r^2 = 0.84$, $n = 32$). Samples over the whole time series from cultures based on medium from humic-rich Lake Tvigölingen (treatments 4-1, 4-2, 4-3, and 4-4) were counted under the microscope since a reliable determination of cell concentration by flow cytometry was not possible because of high background scatter.

For bacterial biomass determination digital images were obtained with a Nikon DXM1200 camera. Between 6 and 10 pictures of each sample were taken to enable analysis of the cell size of at least 150 cells using the software Easy Image Analysis 2000 (Bergström Instrument). Cell volume and carbon content per cell were calculated as described in Eiler et al. (2003).

Cell abundance, biomass, and O_2 utilization were used to calculate bacterial biomass yield (Y_B), maximum intrinsic growth rate (μ), and bacterial growth efficiency (BGE). Y_B was calculated as the product of stationary-phase cell numbers and cellular carbon content. Maximum intrinsic growth rate was calculated as the slope of the linear regression within the interval of linear increase of ln-transformed bacterial abundance plotted as a function of time, including at least three data points. BGE was calculated as $Y_B/(Y_B + BR) \times 100$. A gross estimate of the relative portion of total DOC utilized in the experiment (%DOC) was obtained by dividing Y_B + BR with the DOC concentration in the lake water.

Statistical analysis of functional parameters—Differences in functional parameters among treatments in dependence of the origin of the medium and inoculum, respectively, were tested with two-factorial analysis of variance (ANOVA) and a subsequent Tukey honestly significant difference (HSD) test. Principal components analysis (PCA) was performed on a correlation matrix of normalized data. A two-way multivariate ANOVA (MANOVA) (Pillai's trace test) was performed on the scores of the first two principal components. Treatments inoculated with bacteria from lake 4 were excluded from the analysis, because of the lack of consistent growth and obviously different results. K-means cluster analysis was used to verify the PCA results. K-means clustering is a nonhierachical method often used as a second step to confirm the existence of a certain number of clusters as defined by another method. The procedure is that each data point is associated to one of a predefined number of clusters. All statistical analyses were carried out using Statistica 6.0 (StatSoft).

DNA extraction and t-RFLP analysis—At the end of the experiment we analyzed community composition by t-RFLP. Samples of 1.5 ml were filled into sterile vials and centrifuged at 10,000 $\times$ g for 30 min. The supernatant was removed and another 1.5 ml of water added and the procedure repeated once more. The pellet was stored at -70°C. DNA extraction was done with the DNeasy kit (Qiagen) as described by Lindström and Bergström (2004).

For polymerase chain reaction (PCR) amplification we used the fluoresecently marked bacterial forward primer 27f (Vergin et al. 1998) and the reverse universal primer 519r (Lane et al. 1985). The forward primer was labeled with hexachlorofluorescein (HEX) at the 5′ end (MWG Biotech). For each sample three replicates of 50-μl reactions were set up where each reaction consisted of 0.2 μmol L^{-1} each primer, 200 μmol L^{-1} each dNTP, 0.5 μl of 0.25 U Taq polymerase, and ~10 ng of DNA in 1× PCR reaction buffer (10 mmol L^{-1} Tris-HCl, pH 9, 50 mmol L^{-1} KCL, 0.1% Triton X-100, and 3 mmol L^{-1} $MgCl_2$) (Invitrogen). Samples were amplified using an initial denaturation step at 94°C (3 min), followed by 25 cycles of denaturation at 94°C (1 min), annealing at 55°C (1 min), and an extention at 72°C (3 min). This was followed by a final primer extension at 72°C (7 min). The replicate PCR reactions from each sample were pooled and pseudofragments were eliminated by digesting single-stranded DNA with mung bean nuclease (Egert and Friedrich 2003). The products were subsequently purified and concentrated using the QIAquick PCR purification kit

(Qiagen). Restriction digests were done using the restriction enzymes *Hha*I, *Hae*III, and *Alu*I (Invitrogen). PCR products (200 ng) were digested in 10-μl aliquots according to the instructions of the manufacturer for 16 h at 37°C. The digest was diluted 1 : 10 with Milli-Q water before the separation and detection of HEX-labeled fragments with an ABI 3700 96-capillary sequencer running in GeneScan mode (Applied Biosystems) together with an internal lane standard (GeneScan-1000 ROX PE, Applied Biosystems). Control samples consisting either of nondigested PCR product to control for the quality of the PCR product or Milli-Q water to control for external contamination were included.

Treatments 1.4, 2.4, and 3.4 did not yield good-quality t-RFLP profiles because of insufficient concentrations of DNA in the majority of the replicates, which made it difficult to obtain enough DNA for successful and reproducible PCR reactions. Therefore these samples were excluded from the analysis.

t-RFLP electropherograms were inspected with the free software GenScan View 4 (CRIBI group, http://www.grup.cribi.unipd.it). The t-RFLP patterns obtained from the three different enzymes were pooled for each sample. The data set for subsequent clustering analysis was constructed taking into account peaks with a size of 40–500 base pairs (bp) and a relative peak height >0.5% of the total signal, which reduced the number of peaks to 40–60 per sample. Peaks less than 1.5 bases apart from a larger peak were merged. To account for small differences in running time among samples, peaks with <1 bp difference were considered to be of the same length.

t-RFLP patterns were compared through calculation of a similarity matrix using the Dice similarity coefficient, which calculates the similarity between a pair of samples on the basis of binary data, i.e., taking into account only the presence or absence of peaks. Hence, our analysis was semiquantitative, since we only included the largest peaks (i.e., those comprising at least 0.5% of the total peak height), but treated those peaks identically after the first exclusion step. The matrix was then analyzed by nonmetric multidimensional scaling (NMDS) with three predefined dimensions to reduce the final stress value of the ordination to a value <0.15, which is a postulate for a reliable interpretation of the observed patterns. A two-way MANOVA (Pillai's trace test) was performed on the scores of the three dimensions to test whether the origin of the medium or inoculum (or both) had an effect on composition of bacterial communities. K-means clustering was used to test if there was an overlap between functional and structural clustering. The number of clusters used as input for k-means clustering was chosen on the basis of the results from the ordination and k-means clustering with functional parameters. All statistical analyses were carried out using Statistica 6.0 (StatSoft).

In addition to the analysis of the t-RFLP profiles as outlined in the section above, we performed a second quantitative procedure using PCA based on peak height data. This resulted in a clustering pattern comparable to that of the NMDS analysis, indicating that the general patterns of differences between treatments in dependence of the source of the medium and inoculum, respectively, were independent of the way in which the t-RFLP profiles were analyzed. For reasons of simplicity and clarity, we present only the results obtained from the NMDS analysis based on presence/absence data in this paper.

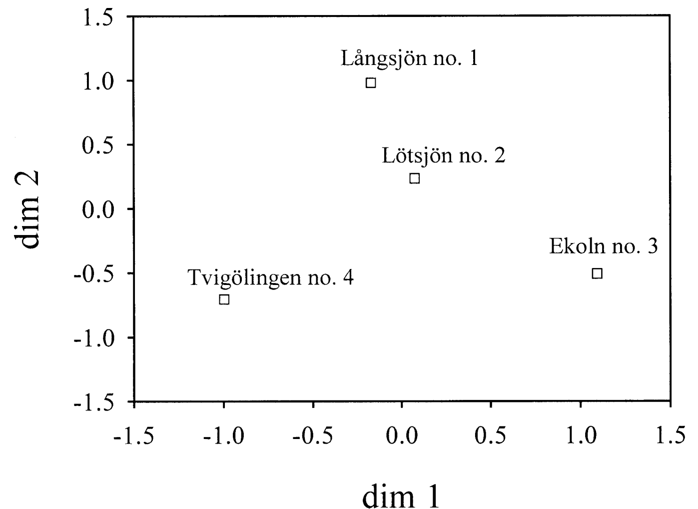

Fig. 1. Nonmetric multidimensional scaling analysis of Dice distance matrices calculated from t-RFLP patterns based on presence/absence data obtained from Långsjön (no.1), Lötsjön (no.2), Ekoln (no.3), and Tvigölingen (no. 4). Stress < 0.001.

Results

Lake characteristics, effects of autoclaving, and controls—Some characteristics of the four lakes are summarized in Table 2. All lakes had close to neutral pH values and high alkalinity. DOC concentration varied widely and was highest in the humic Lake Tvigölingen (Table 2). Bacterial abundance was similar in Långsjön, Lötsjön, and Ekoln, but twice as high in Tvigölingen (Table 2). The lakes differed clearly in bacterial community structure as described by t-RFLP patterns, with Långsjön and Lötsjön having most genotypes in common (Fig. 1). Autoclaving of the 0.2-μm-filtered lake water affected pH, alkalinity, and DOC (Table 3). Even though the DOC concentration was unaffected by autoclaving (this was tested before the experiment, data not shown), the absorbance changed and in all cases the ratio $A_{250} : A_{365}$ decreased, indicating an overall increase in molecular size (Strome and Miller 1978). However, relative differences between different DOC sources were maintained since, for example, lake 1 had the highest and lake 4 the lowest A_{250} : A_{365} ratio both before and after autoclaving, respectively (Table 3).

Alkalinity changed notably only in the case of Långsjön (medium 1), where a clear decrease occurred after autoclaving. This was even visible in the form of carbonate precipitating in the experimental bottles. pH increased in media 2, 3, and 4 (Table 3) and created clear differences between treatments, resulting in media 1 and 4 and 2 and 3 being similar in terms of pH, respectively.

Control cultures that only contained the 0.2-μm-filtered lake water but no inocula remained, with one exception, sterile until the end of the experiment (Fig. 2).

Table 3. Effects of autoclaving on pH, alkalinity, and DOC properties (ratio of absorbance at 250 and 365 nm, $A_{250}:A_{365}$) of the medium.

Lake	pH		Alkalinity (mekv L^{-1})		$A_{250}:A_{365}$	
	Before	After	Before	After	Before	After
1	8.30	8.31	2.94	1.14	14.9	10.3
2	8.01	9.42	1.63	1.79	10.8	7.7
3	7.76	9.33	2.24	2.31	6.3	5.9
4	6.82	8.09	0.48	0.57	4.8	4.2

Functional parameters—Typical batch culture growth curves with an initial lag phase, an exponential growth phase, and a stationary phase were observed in most treatments (Fig. 2). The only exception to this was found in treatments where bacteria from humic-rich Lake Tvigölingen (Inoc 4) were exposed to extraneous media (Figs. 2, 3). In treatments 2.4 and 3.4, no or only retarded and nonregular growth occurred. In treatment 1.4 we observed a prolonged lag phase and lower stationary phase biomass yield compared to the other inocula growing on the same medium (two-way ANOVA, Tukey HSD test, data not shown). When inoculated into the original medium (lake 4), the bacteria from lake 4 exhibited normal growth, similar to the other inocula (Fig. 2). In cultures based on medium 2 and medium 3, pH was high (>9) after autoclaving (Table 3). We observed earlier that bacteria from Lake Tvigölingen growing on glucose as the only carbon source showed a continuous decline in stationary phase biomass when growing over a pH gradient 8–10, with almost no growth occurring in cultures with pH > 9.5 (unpubl. data).

Apart from the inoculum effect connected to bacteria from Lake Tvigölingen, however, functional parameters were affected almost exclusively by the medium (Fig. 3, Table 4; inoculum 4 was excluded from the statistical analysis). There were strong significant effects of the medium on bacterial abundance, biomass yield, growth rates, respiration, growth efficiency, and DOC utilization, whereas there was only a weak significant effect of the origin of the inoculum in one

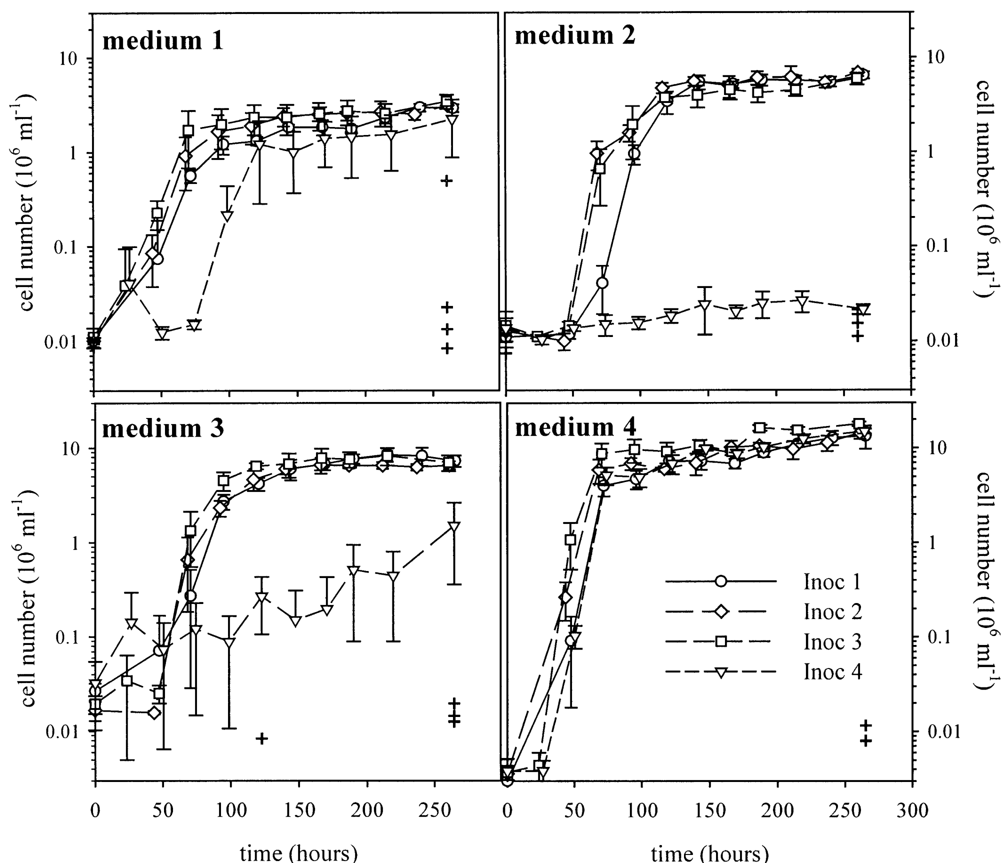

Fig. 2. Growth of bacterial communities in the cultures. Treatments based on the same medium but different inocula are shown together in the same graph. Cell numbers from control cultures (symbolized by crosshairs) are shown for the end of the experiment. All symbols represent mean values ± SD calculated from four replicate cultures.

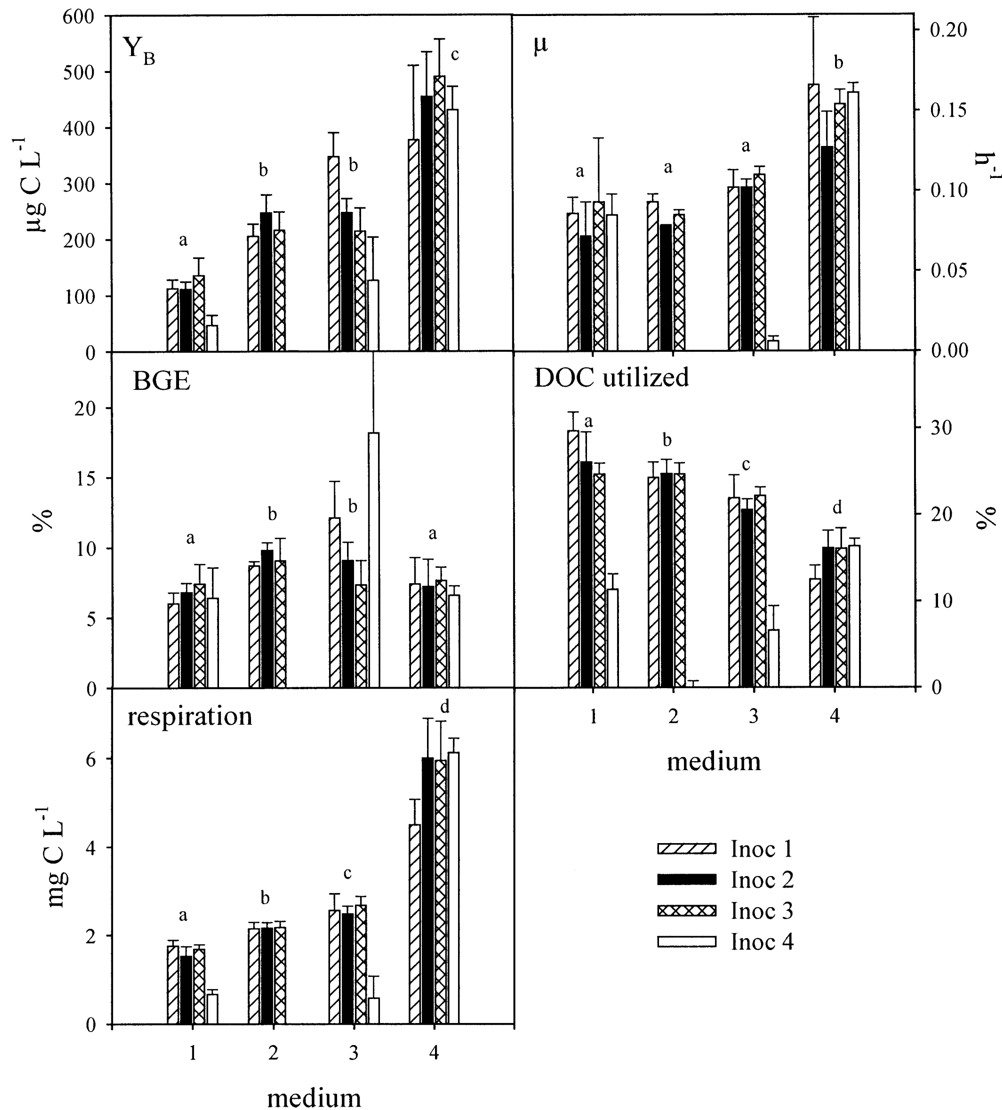

Fig. 3. Functional parameters measured during or at the end of the experiment. Treatments based on the same medium but different inocula are shown together in the same graph. Y_B, bacterial biomass yield; μ, maximum intrinsic growth rate; BR, bacterial respiration; BGE, bacterial growth efficiency; %DOC, percentage of total DOC utilized. All symbols represent mean values ± SD calculated from three or four replicate cultures. Letters on top of the bars indicate results from a Tukey's HSD post hoc test of a two-factorial ANOVA (Table 4). Different letters indicate significant differences ($p < 0.05$) resulting from the origin of the medium as an independent factor. All treatments that received an inoculum from lake no. 4 were excluded from the analysis.

Table 4. p values from a two-way factorial ANOVA on different functional parameters. Treatments receiving an inoculum from lake 4 (Inoc 4) were excluded from the analysis. Significant p values ($p < 0.05$) are printed bold.

	Abund	Y_B	μ	BR	BGE	%DOC
Medium	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Inoc	0.28	0.97	**0.04**	0.17	0.54	0.30
Medium×Inoc	0.09	**0.004**	0.61	**0.03**	**0.01**	**0.037**

Abund, abundance at the final stage of the experiment; Y_B, bacterial biomass yield; μ, maximum intrinsic growth rate; BR, bacterial respiration; BGE, bacterial growth efficiency; %DOC, % of total DOC utilized. Values for Y_B, abundance, and BR were transformed by log (x), and BGE and %DOC were arcsin square-root transformed.

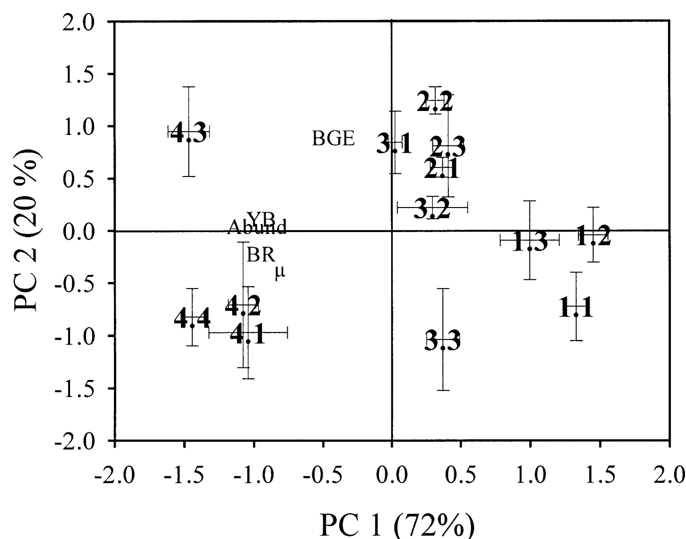

Fig. 4. Principal component analysis (PCA) with functional parameters. The distribution (scores) of treatments along the first two principal components is shown. Loadings of functional variables included in the analysis (Abund, Y_B, BGE, μ, and BR) are shown as well (see Table 4 for abbreviations). All symbols represent mean values ± SD calculated from three or four replicate cultures.

Table 5. Results of k-means clustering analysis with three predefined clusters. Clustering was done for functional variables (abundance, Y_B, μ, BGE, BR) and t-RFLP patterns. Treatments 1.4, 2.4, and 3.4 were excluded from the analysis. For abbreviations and data transformations see Tables 1 and 4. "Genetic" refers to data from t-RFLP analysis of bacterial communities.

Cluster	Functional	Genetic
1	1.1, 1.2, 1.3	1.1, 1.2, 1.3, 2.2, 3.2
2	2.1, 2.2, 2.3, 3.1, 3.2, 3.3	2.1, 2.3, 3.1, 3.3
3	4.1, 4.2, 4.3, 4.4	4.1, 4.2, 4.3, 4.4

of six measured parameters (Table 4). Bacterial abundance, Y_B, and BR were highest in cultures based on medium 4 and lowest in cultures growing in medium 1 (Fig. 3). Media 2 and 3 were intermediate and did not differ significantly from each other (Fig. 3). Maximum intrinsic growth rates (μ) were highest in cultures based on medium 4 and did not differ significantly among the other treatments (Fig. 3). BGE was significantly higher in cultures based on media 2 and 3 compared to those based on media 1 and 4 (Fig. 3). The relative amount of DOC that was utilized during growth in the cultures was significantly lower in cultures based on medium 4 compared to the rest (Fig. 3).

PCA, carried out with functional parameters as variables, grouped the cultures into three apparent clusters along the first axis: medium 1, media 2 and 3, and medium 4 (Fig. 4). Only the source of the medium but not the origin of the inoculum had a significant effect on the clustering of treatments (two-way MANOVA: medium: $F_{6,58} = 18.8$, $p < 0.0001$; inoculum: $F_{4,58} = 0.70$, $p = 0.60$, medium × inoculum: $F_{12,58} = 2.22$, $p = 0.02$). The separation along the first principal component was to a large extent explained by differences in bacterial abundances and biomass yield among cultures and generally in good agreement with the ANOVA results (Fig. 4, Table 4). K-means clustering with a predefined number of three clusters confirmed the existence of three functional clusters (Table 5).

Bacterial community structure—The proportion of the total number of t-RFLP peaks shared between pairs of samples was on average 0.27 ± 0.09 (n = 1,236) between profiles from different treatments and 0.57 ± 0.09 (n = 59) between profiles obtained from replicate cultures. NMDS analysis of t-RFLP profiles (Fig. 5) suggests that the pattern underlying bacterial community structure is rather complex. When plot-

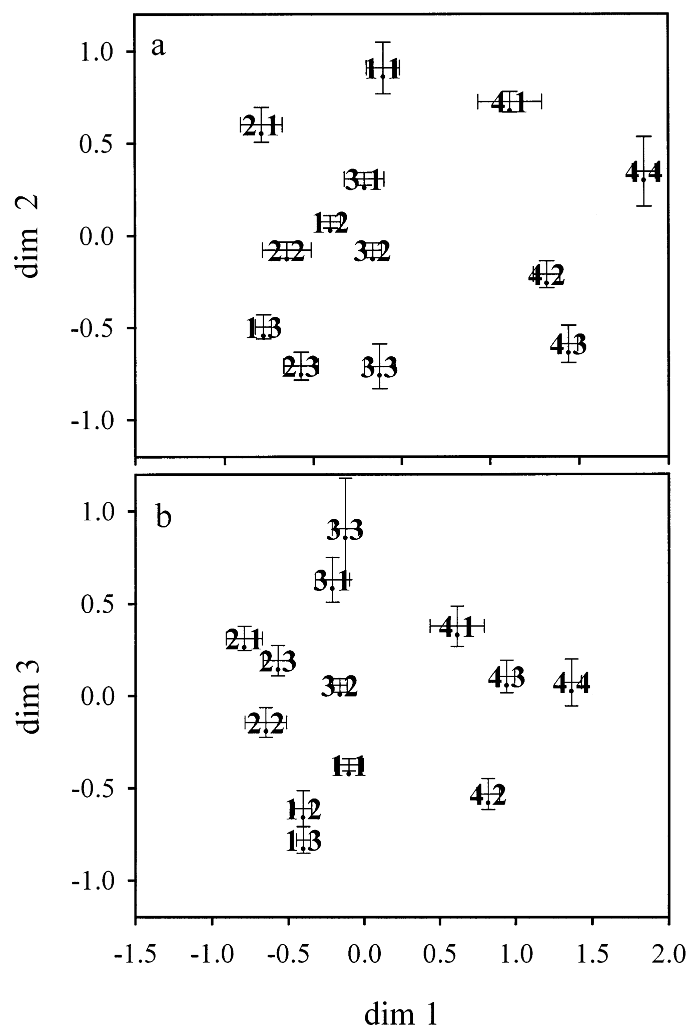

Fig. 5. Results from a three-dimensional nonmetric multidimensional scaling analysis based on a Dice similarity matrix calculated from t-RFLP data using presence/absence data. (a) Dimension 1 versus dimension 2, (b) dimension 1 versus dimension 3, Stress (of three-dimensional model) = 0.125. Three or four replicates were included for each treatment in the analysis and all symbols represent mean values ± SE calculated from replicate cultures. Exclusion of replicates was done because of failure at different steps during the t-RFLP analysis (e.g., insufficient amount of PCR product, evaporation of samples, etc.). Treatments 1.4, 2.4, and 3.4 were completely excluded because PCR amplification did not yield enough material to continue with the subsequent steps in most cases.

ting dimension 1 versus dimension 2, for example, treatments based on medium 4 clearly separate from the other treatment along the first dimension, while there was a separation according to the origin of the inoculum along the second dimension (Fig. 5a). Similar patterns were observed in the two-dimensional space along dimensions 1 and 3, with the only difference that in this case the separation along the *y*-axis (dimension 3) was in accordance with the origin of the medium (Fig. 5b). Both origin of medium and inoculum had significant effects on bacterial community structure (two-way MANOVA: medium: $F_{9,96} = 15.5$, $p < 0.0001$; inoculum: $F_{6,62} = 36.3$, $p < 0.0001$; medium × inoculum: $F_{18,96} = 3.10$, $p < 0.0001$). We performed k-means clustering to test whether similar clustering patterns as for the functional parameters would be observed (Fig. 4). All treatments receiving medium 4 were members of one consistent cluster, whereas the other two clusters were mixed and contained treatments differing in medium, inoculum, or both (Table 5).

In summary, the results suggest that both the origin of the inoculum and medium determined the genetic structure of the bacterial communities.

Discussion

Response of humic lake bacteria to changing conditions—It was surprising to find that bacteria originating from the lake with the highest DOC concentration (Inoc 4) failed to grow (2.4), or grew unsteadily (3.4) or with an extended lag phase (1.4) when exposed to media originating from other lakes (Fig. 2). They grew, however, in the same manner as all other inocula, under ''native'' conditions, i.e., on medium 4 (Fig. 2). The ability of Inoc 4 to grow decreased with increasing pH values of the medium and the strongest effects were observed in treatments with pH > 9. In an independent experiment we found that bacteria from the same lake showed a very similar pattern of growth decrease on glucose in artificial lake water over a pH gradient of 8–10 (unpubl. data). Thus, it seems likely that differences in pH caused the growth pattern shown by Inoc 4. In a previous study, Edling and Tranvik (1996) found that the growth in similar cultures was affected by manipulation of pH, but the response to pH varied among lakes. It has previously been suggested that pH can be a factor regulating bacterial community composition in freshwater systems (Lindström and Leskinen 2002). Soil studies have shown that bacterial communities adapt to drastic changes in pH at varying rates and that this is accompanied by a change in community composition (Pettersson and Bååth 2003).

Lake Tvigölingen (no. 4) is a highly humic forest lake that can be expected to differ not only in terms of organic matter quantity but also in composition and quality compared to less humic lakes (Tranvik 1990). Divergent relative differences of absorbance at different wavelengths reflect qualitative differences in DOM. The ratio of absorbance at 250 and 365 nm is suggested to supply information about the size distribution of different components (Strome and Miller 1978). The ratio $A_{250} : A_{365}$ was lowest in Tvigölingen, indicating that, in comparison to the other lakes, a relatively high fraction of the DOC consisted of larger molecules. Thus, the inability of Inoc 4 to adapt to new conditions in the cultures might have been a consequence of (1) high pH or (2) lacking the ability to cope with the differently composed DOM pool in the more dilute environments, or both. However, if ''everything was everywhere'' there should be alkaliphilic bacteria in the inoculum also from this lake, able to utilize components of the new DOM pool, profiting from the change in conditions. The t-RFLP profile obtained from one of the replicates of treatment 1.4, where community growth was observed after an extended lag phase, although biomass levels remained lower compared to other cultures grown in the same medium, showed that the community shared most of the peaks with other treatments. This indicates that in this case the same or similar genotypes were selected, but that the adaptation times were much longer.

Influence of medium and origin of inoculum on functional properties of bacterial communities—Except for the impeded or delayed growth when bacteria originating from humic-rich lake Tvigölingen were confronted with ''non-native conditions'', there were no or only very weak effects of the origin of the inoculum on functional properties of bacterial communities. In contrast, the medium had strong significant effects on all parameters we measured (abundance, biomass yield, respiration, bacterial growth efficiency, percentage of total DOC utilized). Hence, functioning was rather independent of the ''history'' of bacterial communities used for inoculation, suggesting that they are able to adapt to the conditions brought along with the new DOM pools of the different media. Similar observations have been made in other studies (Tranvik and Höfle 1987; Gasol et al. 2002; Findlay et al. 2003). Tranvik and Höfle (1987), for example, inoculated clear water and humic water batch cultures in all combinations and found that patterns of growth were influenced only by the carbon source but not by the inoculum. Similar results were also found in cultures based on marine water from two sites differing in trophic state (Fuchs et al. 2000).

PCA and subsequent k-means clustering based on all functional parameters measured revealed three distinct functional clusters: medium 1, media 2 and 3, and medium 4 (Fig. 4). Generally, medium 2 and 3 were similar in terms of functional properties. This is not surprising since the underlying lakes (Lötsjön and Ekoln) are quite similar in terms of chemical characteristics (Table 2). They both represent the typical lake type in the study area (moderately stained, high alkalinity, mesoeutrophic). Långsjön (medium 1) differs from these two lakes because it is oligotrophic and has a low DOC concentration due to high groundwater input (Table 2). Tvigölingen (medium 4) is the lake with the most pronounced differences (Table 2) and it is interesting that the cultures based on water from this lake were most distinct in terms of functional properties.

Influence of the medium and origin of inoculum on bacterial community composition—We hypothesized that cultures growing in the same media should be similar in bacterial community composition—independently of the origin of the inoculum. Instead we found a complex pattern indicating that the medium as well as the initial composition of

the inoculum influenced the structure of bacterial communities (Fig. 5). Burkert et al. (2003) showed that the same members of the *Polynucleobacter necessarius* lineage dominated within the bacterial assemblages enriched in cultures based on acidotrophic humic water, independently of the source of bacteria used for inoculation. In accordance with our results, however, Kirchman et al. (2004) could show that both the medium and the inoculum influenced the abundance of major bacterial groups (alpha-, beta-, and gamma-Proteobacteria and *Cytophaga*-like bacteria) in river water cultures. Other laboratory as well as field studies have also shown that the quantity and quality of the organic matter have strong impacts on the community composition of aquatic bacteria (Pinhassi et al. 1999; Crump et al. 2003; Eiler et al. 2003). Moreover, specialization for the consumption of certain organic molecules occurs among bacterial groups (Cottrell and Kirchman 2000; Covert and Moran 2001).

In our study, both NMDS and k-means cluster analysis (Fig. 5, Table 5) showed that medium 4 selected for similar though not identical bacterial communities from all inocula. Since medium 4 originated from polyhumic water where DOM of high molecular weight dominates (Tranvik 1990), it seems possible that the majority of the selected bacteria was able to utilize these substances. As mentioned above, our data suggest that the source of the medium and the inoculum both separately influence the structure of the bacterial communities developing in the cultures. This indicates that bacterial communities might be composed of two ''physiological'' groups of bacteria. We suggest that the shaping effect of the inoculum on the bacterial community composition reflects taxa that are metabolically versatile and able to adapt quickly when faced with a new DOM pool. Similarly, the effect of the origin of the medium on the developing bacterial community reflects bacteria that are specifically adapted for growth under certain conditions and hence get selected under the force of these environmental conditions. Among those might be taxa especially adapted to the utilization of certain substrates, growth at certain pH levels, etc. This scenario corresponds well with what has been suggested for natural bacterioplankton communities. They are believed to comprise a combination of persistent populations of generalists present throughout the years and transient populations fluctuating in presence and abundance depending on seasonal variations in environmental conditions (Lindström 1998; Crump et al. 2003; Stepanauskas et al. 2003).

Coupling between functioning and community composition—Several studies have found a close coupling between bacterial community composition and functioning in the sense that communities differing in terms of function harbor distinctly different communities (Franklin et al. 2001; Findlay et al. 2003). In contrast to these previous investigations, our study allows us to conclude that functional similarity under similar environmental conditions may not simply be a result of similarly composed bacterial communities. Others have also demonstrated that structurally highly dynamic communities can maintain a stable ecosystem function (Fernandéz et al. 1999). In another study, biofilm communities responded to nitrogen additions by changes in functioning, whereas there was no parallel change in community structure (Findlay and Sinsabaugh 2004). Recently, Kirchman et al. (2004) found that in bacterial growth experiments with water and bacteria from different parts of the Hudson River, ectoenzyme activities were mostly affected by the source of the water, whereas the bacterial community structure was affected by both the origin of the water and the inoculum. We found differently composed communities under functionally similar conditions. Hence, our findings support that the coupling between functioning and composition of bacterial communities is not necessarily tight and that natural bacterial communities exhibit considerable functional redundancy. Nevertheless, our results also pointed to the existence of a ''humic cluster,'' i.e., those bacteria growing in medium 4 that was distinctly different in terms of community functioning and composition (Table 5).

A crucial step in this study is the clear separation of medium and inoculum effects, i.e., lake water bacteria must be absent from the media before inoculation. Sterile filtration would affect the DOM in the lake water little, but would not guarantee sterility since small bacteria are able to pass through the pores of the filters and therefore contaminate the medium. For instance, Hahn (2003) successfully used the water passing 0.2-μm filters for the cultivation of small natural bacteria. Hence, we had to autoclave the water twice, at the expense of resemblance to natural conditions (Table 3). Autoclaving affected pH and alkalinity as well as DOC properties, as indicated by the $A_{250}:A_{365}$ ratio (Table 3). Even though DOC concentrations did not change and relative differences in $A_{250}:A_{365}$ ratios among media remained approximately stable even after autoclaving, we cannot exclude that autoclaving affected the quantity and composition of the labile DOM pool and other factors that may affect the bacterial community, such as trace metal availability. Biomass yields in this study were in all cases within 50% of values predicted from a regression of yield as a function of DOC concentration in similar cultures on the basis of 0.2-μm-filtered water without autoclaving and no inorganic nutrient additions (Tranvik 1988). The average amount of labile DOC was estimated to be 20% ($\pm$7%) and therefore in the range of what is observed in other lake water studies (del Giorgio and Davis 2003). Hence, despite autoclaving, the general level of DOC bioavailability remained similar to what has been found under nonautoclave conditions. To conclude, we sacrificed some of the resemblance of the experimental conditions to the natural situation, but on the other hand this allowed us to clearly separate inoculum and medium effects to test the specific questions asked.

In this study we investigated general functions (biomass yield, growth rate, respiration etc.) and community composition patterns detectable by t-RFLP. The results indicate that major functions related to the turnover of organic carbon (respiration, DOC consumption, biomass production) are weakly coupled to the composition of the bacterial communities. It remains to be seen whether more specific functions (e.g., the degradation of specific organic compounds) or functions related to the cycling of other elements (e.g., phosphorus) would yield a similar pattern. Moreover, further insight into the response in community composition may be revealed in the future by methods with a higher discrimi-

nating power than t-RFLP (Casamayor et al. 2002), and by methods that do not depend on PCR with its potential biases (von Wintzingerode et al. 1997). Finally, there may be changes in community composition that were not detected because of possible nonuniversality of the used primers (e.g. Forney et al. 2004). Despite these limitations, we found changes in community composition that were only partly coupled to differences in functioning when bacteria were allowed to grow at the expense of DOM from different waters.

To summarize, we found that the functional performance of bacterial communities growing in batch cultures differing in the origin of the medium and the inoculum was dependent on the type of medium but in most cases not on the origin of the bacterial inoculum. Functional changes were, however, only partly paralleled by changes in community composition. Cultures shared bacterial taxa as a result of receiving the same medium or inoculum, indicating that bacterial communities are comprised of populations of generalists that can grow under most conditions as well as populations with the life strategy of specialists.

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