

Influence of dissolved organic matter source on lake bacterioplankton structure and function – implications for seasonal dynamics of community composition

Emma S. Kritzberg¹, Silke Langenheder², & Eva S. Lindström^{2,3}

¹Department of Ecology/Limnology, Lund University, Lund, Sweden; ²Department of Limnology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden; ³Département des sciences biologiques, Université du Québec à Montréal, succursale Centre Ville, Montréal, Québec, Canada

Correspondence: Emma S Kritzberg, Sturegatan 9b, S-211 50 Malmö, Sweden. Tel.: +46 0 40 6118844; fax: +46 0 46 2224536; e-mail: emma.kritzberg@limnol.lu.se

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Abstract

It has been suggested that autochthonous (internally produced) organic carbon and allochthonous (externally produced) organic carbon are utilized by phylogenetically different bacterioplankton. We examined the relationship between the source of organic matter and the structure and function of lake bacterial communities. Differences and seasonal changes in bacterial community composition in two lakes differing in their source of organic matter were followed in relation to environmental variables. We also performed batch culture experiments with amendments of various organic substrates, namely fulvic acids, leachates from algae, and birch and maple leaves. Differences in bacterial community composition between the lakes, analysed by terminal restriction fragment length polymorphism, correlated with variables related to the relative loading of autochthonous and allochthonous carbon (water colour, dissolved organic carbon, nutrients, and pH). Seasonal changes correlated with temperature, chlorophyll and dissolved organic carbon in both lakes. The substrate amendments led to differences in both structure and function, i.e. production, respiration and growth yield, of the bacterial community. In conclusion, our results suggest that the source of organic matter influences community composition both within and among lakes and that there may be a coupling between the structure and function of the bacterial community.

Introduction

There is growing evidence for the significance of allochthonous organic carbon in sustaining the metabolism of aquatic bacteria. In many systems, an imbalance of internal primary production and bacterial production and respiration indicates the importance of allochthonous organic matter to heterotrophic bacteria (del Giorgio *et al.*, 1997; Karlsson *et al.*, 2002). Direct analysis of the carbon (C) source by stable isotope analysis has also demonstrated the importance of allochthonous C for the production of bacterial biomass (Coffin & Cifuentes, 1999; Kritzberg *et al.*, 2004). Nevertheless, there are indications that the bacterial communities in lakes utilize autochthonous dissolved organic carbon (DOC) preferentially to and also more efficiently than allochthonous C (Kritzberg *et al.*, 2004, 2005, in press). The consequence of this can be that, although bacteria utilize large amounts of terrestrially derived C, this C is only inefficiently transferred to organisms on higher trophic levels.

The bacterioplankton is not a homogenous population with coherent ecology and physiology, and it has been shown that different groups of bacteria exhibit different capabilities for utilizing qualitatively different substrates (Cottrell & Kirchman, 2000). Hence, the composition and source of the organic matter might influence the composition of the bacterial community, as a result of differences in the growth rates of different groups of bacteria on different substrates. Such differences in community structure could explain why we observe differential uptake and utilization of autochthonous and allochthonous C when studying the processing of C at the level of the total bacterial community.

The application of techniques from molecular biology has greatly improved our understanding of bacterial communities in the environment by allowing the identification of uncultivated bacteria. Particularly interesting are recent studies that combine molecular techniques and biogeochemistry to explore relationships between bacterial community structure, function, and the cycling of dissolved

organic matter (DOM) in aquatic systems. From such studies we have learnt that utilization of high and low molecular weight DOM (Weinbauer & Höfle, 1999; Cottrell & Kirchman, 2000; Covert & Moran, 2001) and enzyme activity (Weinbauer & Höfle, 1999; Kirchman *et al.*, 2004) differ among major phylogenetic groups. Furthermore, changes in phytoplankton community composition can produce a response in bacterioplankton species composition (Pinhassi *et al.*, 2004), possibly as a result of the differential release of organic matter from the algae (Van Hannen *et al.*, 1999). Thus, the quantity and quality of DOM has been suggested as one of several important factors structuring the bacterial community in lakes (e.g. Methé & Zehr, 1999; Crump *et al.*, 2003; Stepanauskas *et al.*, 2003; Jardillier *et al.*, 2004). Moreover, in a study of 13 Wisconsin lakes (Yannarell & Triplett, 2004), the degree of net heterotrophy or net autotrophy appeared to be one of the structuring factors of the bacterial communities, suggesting that the DOM of autochthonous origin and that of allochthonous origin are processed by phylogenetically different bacteria.

The aim of this study was to determine whether the source of organic substrate can influence lake bacterioplankton community structure and function. Two small forest lakes differing considerably in their source of organic matter were studied. Tuesday Lake was slightly net-heterotrophic over the season, and the allochthonous contribution to bacterial C was estimated to be 78% (Kritzberg *et al.*, in press). In contrast, Peter Lake was subject to nutrient enrichment, promoting algal production, and was subsequently net-autotrophic. The bacterioplankton derived on average 54% of their production from phytoplankton-produced C (Kritzberg *et al.*, in press). Thus, bacteria in these two lakes depended largely on DOM of different origin and composition. In addition, the relative input of allochthonous and autochthonous DOC in Peter Lake changed over time towards more autochthonous C. We analysed the seasonal changes in bacterial community composition of the lakes by terminal restriction fragment polymorphism (t-RFLP) in relation to environmental variables of the lakes. In addition, we performed batch culture experiments in which water from Tuesday Lake was amended with DOM leachates from birch and maple leaves, algae and also fulvic acids.

Methods

Lake description and sampling

The study consisted of two parts. The first part aimed to observe the seasonal dynamics in bacterial community composition in two lakes that differed with respect to autochthonous and allochthonous carbon loading, and to explore how it related to internal conditions of the lakes.

The second part was a batch culture experiment in one of the lakes, where we wanted to see if amendments with different DOM substrates promoted different bacterial communities with differing functions.

For the first part of the study, the lakes Tuesday and Peter were sampled weekly from the end of May until late July 2002. The lakes lie on the grounds of the University of Notre Dame Environmental Research Center (UNDERC, 89°32'W, 46°13'N) in northern Wisconsin and are located < 0.5 km apart. On a broad scale, the lakes are in the same drainage basin and both lakes are seepage lakes (Cole & Pace, 1998). Tuesday and Peter lakes are small kettle lakes (0.9 and 2.5 ha) surrounded by wetlands and forest dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula lutea*) and balsam fir (*Abies balsamea*). Both lakes have fish communities dominated by planktivorous minnows suppressing large zooplankton. The lakes differ in dissolved organic carbon (DOC) content and water colour; averages for the 2001 field season were 8.4 and 4.5 mg L⁻¹ and 3.5 and 1.3 m⁻¹ (absorbance at 440 nm) for Tuesday and Peter Lake, respectively.

The lakes are oligotrophic and similar with regards to epilimnetic phosphorus concentration (surface means from May to August of 2001 were 10 and 8 mg P m⁻³) and chlorophyll *a* (5.3 and 4.1 mg m⁻³). However, from June 3 and onwards additions of liquid fertilizer (NH₄NO₃ and H₃PO₄; N:P = 25) were made to Peter Lake in order to make it eutrophic. An initial addition of 23 mg P m⁻² and 265 mg N m⁻² was made on June 3. From June 10 daily additions corresponding to a P loading rate of 3.3 mg P m⁻² day⁻¹ were made.

For bacterial community composition (BCC), water was collected with a 2 m-long tube sampler and transported to the lab at *in situ* temperature. For each lake, three sterile microcentrifuge tubes were filled with 1.5 mL subsamples from the collected water volume and centrifuged at 17 000 g for 30 min (Øvreås *et al.*, 1997). The supernatant was withdrawn and the bacterial pellets were stored in the centrifuge tubes first at -20 °C (for up to 2 months) and then at -80 °C. This material was subsequently used for DNA extraction (Lindström & Bergström, 2004). Concurrently with the samples used for BCC, temperature was recorded and samples were taken for analysis of pH, total phosphorus, total nitrogen, DOC, water colour, partial pressure of carbon dioxide, bacterial abundance, bacterial production and chlorophyll *a*.

Experimental set-up

Four DOM substrates, namely algae, birch, maple and fulvic acids, were prepared. Freeze-dried *Chlorella vulgaris* (Life-stream Research, Auckland, New Zealand) were dissolved in autoclaved reverse osmosis (RO) water and subsequently

ultra-sonicated at 35 000 Hz for 2×30 min. Particulate material was removed by centrifugation and subsequent GF/F filtration (Whatman, Brentford, UK; nominal pore size $0.7 \mu\text{m}$). The final DOC concentration of the algal leachate was 1257 mg CL^{-1} . Leaves of yellow birch (*Betula lutea*, 250 g wet weight) and sugar maple (*Acer saccharum*, 250 g wet) were rinsed with tap water and subsequently leached in autoclaved RO water (final volume water and leaves: 1 L). The leaching took place in darkness at 4°C for 48 h. The concentrations of the leachates were 1619 and 1274 mg CL^{-1} , respectively. Freeze-dried Laurentian fulvic acids (Fredriks Research Products, Amsterdam, the Netherlands) were dissolved in autoclaved RO water to a concentration of 1263 mg CL^{-1} . The four substrates (A: algae, B: birch, M: maple, F: fulvic acids) were filtered through GF/F filters and frozen until the start of the experiments.

Water was collected from Tuesday Lake on 17 July as described above. The water was filtered through precombusted AE filters (Gelman) and autoclaved $0.2 \mu\text{m}$ filters, and subvolumes of 1 L were transferred to 1.2 L glass bottles. DOM substrates (A, B, M and F) corresponding to a DOC increase of *c.* 10 mg CL^{-1} (a doubling of the ambient DOC concentration in Tuesday Lake) were added to triplicate glass bottles with Tuesday water. Immediately before the amendments, the DOM substrates were filtered through autoclaved $0.2\text{-}\mu\text{m}$ filters three times in order to remove all bacteria before experimental treatments. To check that no bacteria were added with the substrates, negative controls with autoclaved lake water and additions of substrates were kept under conditions identical to those of the experimental treatments. These control cultures remained sterile until the end of the experiment. The addition of substrates caused changes in pH, and $0.5 \text{ N H}_2\text{SO}_4$ and 0.5 N NaOH were added to readjust to the original pH (6.2). Finally, the various growth media were inoculated with 100 mL of GF/D-filtered (Whatman, nominal pore size $2.7 \mu\text{m}$) Tuesday water. GF/D filtration decreased bacterial abundance to 93% of the natural abundance and removed virtually all bacterial grazers. The 12 (4×3) bottles were then sealed with gas-tight rubber septa and incubated in darkness at *in situ* temperature (25°C).

After 2 h of incubation, a 60 mL sample was withdrawn from each bottle with a syringe and replaced by an equal volume of N_2 -gas. The sample was used for initial measurements of DOC and dissolved inorganic carbon concentrations. The same sampling procedure was repeated after 63 h. After that sampling, the bottles were opened and samples for BCC were withdrawn as described above. To allow for comparison with the batch cultures, the GF/D-filtered inoculum was sampled for BCC in connection to the inoculation (t_0 sample).

Triplicates of 300-mL BOD bottles (a total of 12), containing the same proportions of $0.2 \mu\text{m}$ filtered lake

water, DOM substrate and GF/D-filtered lake water, were incubated parallel to the 1.2 L batches. The BOD bottles were sampled six times over the 63 h, in order to follow bacterial production.

Accumulated bacterial production (BP) in the 300 mL BOD bottles and accumulated dissolved inorganic carbon (bacterial respiration; BR) in the 1.2 L bottles were used to calculate bacterial growth efficiency (BGE) [$\text{BGE} = \text{BP}/(\text{BP} + \text{BR})$]. The relative proportion of DOC utilized in the experiment was obtained by dividing accumulated $\text{BP} + \text{accumulated dissolved inorganic carbon}$ by the initial DOC concentration.

Analytical methods

pH was measured with an Orion digital meter with two-point calibration and electrodes with automatic temperature compensation. Dissolved inorganic carbon and partial pressure of carbon dioxide ($p\text{CO}_2$) were measured using a Shimadzu GC-8AIT (thermal conductivity detector, Shimadzu Scientific Instruments, Columbia, MO) gas chromatograph. Dissolved inorganic carbon was determined using the method of Stainton (Stainton, 1973), in which headspace from acidified samples is injected into the gas chromatograph. $p\text{CO}_2$ was determined using the method of Cole and others (Cole *et al.*, 1994), in which 2 L of water is equilibrated with 60 mL of air in a polycarbonate bottle. The equilibrated headspace gas is then drawn into 20 mL syringes and transported to the lab for analysis in the gas chromatograph (GC). DOC was measured on GF/F-filtered samples using a Shimadzu TOC-5000 carbon analyser. GF/F filtrates were also used for measuring absorbance at 250 (A_{250}) and 440 nm (A_{440}). Water colour was expressed as wavelength-specific absorption coefficients ($a_{250, 440} = 2.303A_{250, 440}/l$, where l is the optical path-length in metres). Molar absorption coefficients were calculated as $\varepsilon_{250, 440} = a_{250, 440}/C$, where C is the concentration of DOC in mM. ε_{250} was considered an indicator of the contribution of small molecules, and ε_{440} an indicator of the contribution of large molecules enriched in chromophores to the DOC pool (Strome & Miller, 1978). For the seasonal data, total phosphorus (TP) was measured on a Lachat AE autoanalyser (Lachat Instruments, Milwaukee, WI) after persulfate digestion using the molybdate blue method. For total nitrogen (TN), unfiltered water was digested in basic persulphate (D'Elia *et al.*, 1977), and the resulting nitrate was measured by the sulfanilamide method modified for an Alpkem autoanalyser. For the batch cultures, contents of organic and inorganic P and N was measured on GF/F filtrates with a Technicom, autoanalyser. Chlorophyll *a* (Chl *a*) samples were filtered onto Whatman GF/F filters, which were frozen, extracted in methanol, and the concentration determined fluorometrically (Carpenter *et al.*, 1996).

BP was estimated by measuring incorporation of ^3H -leucine (Kirchman, 1993) using microcentrifuge tubes as described by Smith & Azam (1992). Water samples (1.5 mL, four replicates and 1 killed control) were incubated with 60 nM final concentration of ^3H -leucine ($42.5 \text{ Ci mmol}^{-1}$) at *in situ* temperature for 45 min. The incubation was terminated with 30 μL of 50% trichloroacetic acid (TCA). The samples were then centrifuged at 17 000 g for 10 min and the pellet rinsed with 1.5 mL of 5% TCA. After addition of 0.5 mL scintillation cocktail (Scintiverse BD, Fisher Scientific, Hampton, NH), samples were mixed vigorously and ^3H -activity was measured with a Beckman LS 6500 scintillation counter. BP was calculated according to Smith & Azam (1992).

Bacterial community composition was analysed by t-RFLP. DNA extraction of the pellets stored in microcentrifuge tubes was carried out using the DNeasy kit (Qiagen, Hilden, Germany) as described by Lindström & Bergström (2004). Reaction mixtures for PCR amplification contained each primer (27f; Vergin *et al.*, 1998 and 519r; Lane *et al.*, 1985) at a concentration of 0.2 μM , each deoxynucleoside triphosphate at a concentration of 200 μM , 3 mM MgCl_2 , 0.5 μL 1% BSA, 0.25 μL Taq DNA polymerase and approximately 10 ng of DNA in PCR reaction buffer (10 mM Tris-HCl, pH 9, 50 mM KCl and 0.1% Triton X-100). The forward primer used was labelled with hexachlorofluorescein (HEX). DNA amplification was done in a PCR cycle containing an initial denaturation step at 94 °C for 3 min followed by 25 cycles of: 1 min at 94 °C (denaturation), 1 min at 55 °C (annealing) and 1 min at 72 °C (extension), and a final extension at 72 °C for 7 min. Control samples consisting of Milli-Q water to control for external contamination were included. After the amount and quality of the PCR product was checked on a 1% agarose gel in TBE buffer, reaction products from three PCR runs (final volumes of 50 μL) were pooled and pseudo-fragments eliminated by digesting single-stranded DNA with mung bean nuclease (Egert & Friedrich, 2003). Immediately after nuclease treatment, the products were purified and concentrated using the Qiagen PCR purification kit (Qiagen) following the standard protocol.

Aliquots of amplified 16S rRNA gene (5 μL , 100 ng) were digested with restriction enzymes *Hha*I, *Hae*III and *Rsa*I in separate tubes (Invitrogen, Carlsbad, CA) at 37 °C for 16 h, and the reaction was stopped by heating for 15 min at 85 °C. Digests were then separated, and HEX-labelled fragments detected with an ABI 3700 96-capillary sequencer running in GeneScan mode (Applied Biosystems, Foster City, CA) together with an internal lane standard (GeneScan-1000 ROX PE, Applied Biosystems). One of the replicates for the B-amended batch cultures did not yield good-quality t-RFLP profiles. This was caused by insufficient PCR products, which resulted from evaporation in one of the PCR

reactions. Therefore this replicate was excluded from the analysis.

Terminal restriction fragment polymorphism electropherograms were inspected using the free software GeneScan View 4 (CRIBI group, <http://bmr.cribi.unipd.it>). The data set for subsequent analysis was constructed including peaks with a size of 40–500 bp and a relative peak area of > 1% of the total signal. Peaks less than 1.5 bases from a larger peak were merged. Peaks with > 1 bp difference were considered to consist of fragments of different length.

Analysis of t-RFLP fingerprints

For each sample, the community was represented by a t-RFLP pattern constructed by stacking the three individual patterns corresponding to three enzyme digestions. We constructed a binary matrix that considered the presence or absence of individual t-RFLP peaks (t-RFs). This matrix was used to calculate a distance matrix using the Jaccard similarity coefficient (Magurran, 1988). If the coefficient is 0, the two samples are exactly the same. If the coefficient is 1, the two samples have no t-RFs in common. The Jaccard matrix was used to construct a nonmetric multidimensional scaling (NMDS) diagram (Statistica Statsoft, Tulsa, OK). NMDS is an ordination method that reduces the t-RFLP pattern to a point in two-dimensional space. In the diagram, more similar samples are closer than less similar samples. This analysis is qualitative in the sense that only the presence or absence of peaks is considered. In order to include quantitative information, we also performed principal component analysis (PCA) on covariance matrices based on t-RFLP peak-area data.

Statistical analysis

Pearson correlation coefficients (r) and probability values (p) were calculated to check for colinearity among the measured environmental variables. Differences in community composition similarity, based on Jaccard coefficients, were tested using ANOVA, followed by Tukey's *post hoc* test if there were more than two groups to compare. Associations between environmental variables and BCC, i.e. dimensions obtained from NMDS and principal components obtained from PCA of t-RFLP data, were described by Pearson correlation coefficients. Null distributions for the Pearson coefficients were generated by randomly reshuffling the elements of the similarity matrix (bootstrapping; Resampling Stats, Excel add-in). Comparing the Pearson coefficients with the null distributions allowed determination of the likelihood that the observed magnitude was the result of chance. Only principal components with eigenvalues greater than 1 were used for further analysis. Associations between individual t-RFs and environmental variables were also

investigated by Pearson correlation and regular tests of significance.

Numbers of bacterial growth efficiency were calculated from measurements of BP and BR that were made from different bottles, and in that sense do not represent true replicates. ANOVA and Tukey's *post hoc* test were used to test for differences in DOC concentration and bacterial response in the different batch culture treatments. In addition, a PCA was performed on a correlation matrix of normalized data. After normalizing for DOC concentration in the respective treatments, BP and BR were log-transformed and BGE and % DOC utilization were arcsine-transformed to meet conditions of normality. NMDS and PCA patterns of bacterial function and community composition were evaluated by K-means cluster analysis. This procedure attempts to identify a predefined number of relatively homogeneous groups. The clustering is then evaluated by ANOVA. All statistical analyses, except for bootstrapping, were performed using SPSS 10 software for Macintosh (SPSS Inc., Chicago, IL).

Results

Temporal variation

Some characteristics and temporal changes of the two lakes are shown in Fig. 1. For the more humic Tuesday Lake, temperature, BP and $p\text{CO}_2$ increased over the sampling

season while Chl *a* decreased (Fig. 1, Table 1). Thus BP was positively correlated with temperature, while there was a negative correlation between BP and Chl *a* (Table 1). Moreover, CO_2 was supersaturated during the whole period, and there was a strong positive correlation between

Table 1. Correlation matrix for environmental variables in Tuesday and Peter Lakes

Tuesday Lake	Temperature	pH	DOC	Chl <i>a</i>	BP	$p\text{CO}_2$
DOY	0.89**	-0.57	0.66	-0.85**	0.90**	0.86**
Temperature		-0.22	0.67*	-0.85**	0.75*	0.63
pH			-0.21	0.46	-0.68*	-0.78*
DOC				-0.73*	0.42	0.43
Chl <i>a</i>					-0.67*	-0.73*
BP						0.92***
Peter Lake						
DOY	0.92**	0.604	0.83**	0.76*	0.89**	-0.07
Temperature		0.53	0.63	0.70*	0.72*	0.02
pH			0.80**	0.84**	0.55	-0.78*
DOC				0.69*	0.85**	-0.49
Chl <i>a</i>					0.67*	-0.46
BP						-0.21

The numbers are Pearson coefficients and the degree of significance is indicated with asterisks:

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

DOY, day of year; DOC, dissolved organic carbon; BP, bacterial production.

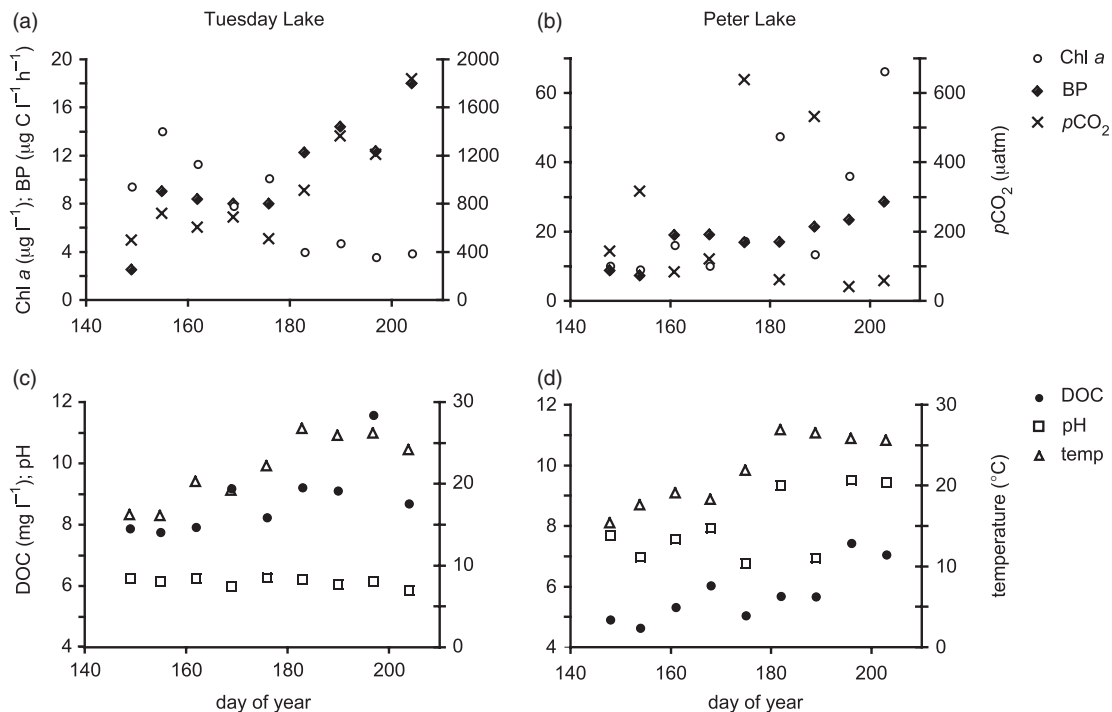


Fig. 1. Time series of selected constituents in the epilimnion of Tuesday and Peter lakes. (a) and (b) Chlorophyll, bacterial production and partial pressure of carbon dioxide. (c) and (d) Dissolved organic carbon, pH and temperature.

BP and $p\text{CO}_2$ and a negative correlation between Chl *a* and $p\text{CO}_2$ (Table 1). This indicates that bacterial activity was a strong factor influencing $p\text{CO}_2$, while primary production may have decreased partial pressure of CO_2 in Tuesday Lake.

In manipulated Peter Lake, temperature, DOC, Chl *a* and BP increased over the period of sampling. In contrast to the pattern in Tuesday Lake, BP and Chl *a* covaried (Fig. 1, Table 1). The positive correlation between BP and Chl *a* may have been the result of the dependence of BP on C derived from phytoplankton production, but it may also be a result of co-dependence on another environmental factor; for example, BP and Chl *a* were positively correlated with both temperature and DOC (Table 1). Partial pressure of CO_2 was highly variable in Peter Lake, from clearly undersaturated to supersaturated levels. The large fluctuations in $p\text{CO}_2$ coincided with a very variable pH.

Upon digestion with the *Hae*III, *Hha*I and *Rsa*I restriction enzymes, 79, 98 and 125, different t-RFLP fragments were produced from the 20 samples. No peak was found in all samples. Thirteen peaks were found throughout the summer in Tuesday Lake (4%), whereas in Peter Lake only one peak occurred in all samples. All samples in Tuesday Lake had a similarity index in t-RFLP patterns above 29%, and the average similarity of all samples in Tuesday Lake was 44%. In Peter Lake, the minimum similarity index was 19%. In addition, the average similarity was lower than in Tuesday Lake (32%, $P < 0.001$, Tukey's *post hoc* test). The similarity within lakes was higher than that between lakes (average 24%, $P < 0.001$, Tukey's *post hoc* test). This is visualized in the NMDS plot (Fig. 2), which also reveals a seasonal succession that was more pronounced in Peter than in Tuesday Lake. Distances in the NMDS ordination reflect

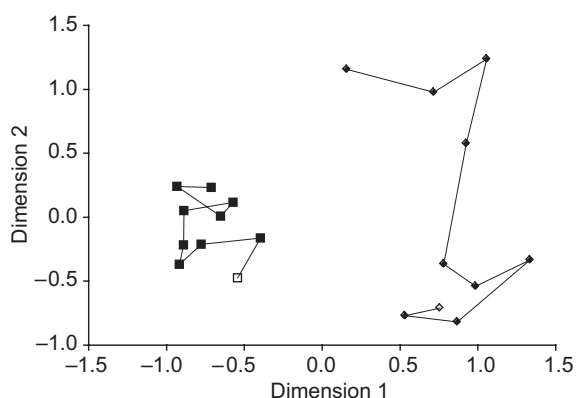


Fig. 2. Nonmetric multidimensional scaling of the Jaccard matrix calculated from terminal restriction fragment polymorphism patterns in Tuesday (squares) and Peter (diamonds) lakes during the summer. Stress = 0.12. Open symbols represent the results from the first sampling date.

the magnitude of dissimilarity in t-RFLP pattern relatively well, as indicated by the goodness of fit (0.12) of the stress value for the ordination with two dimensions (Clarke, 1993). The first NMDS axis clearly separated samples from the two lakes at all times. This axis was correlated with water colour, DOC, TP, TN and pH (Table 2), i.e. the variables that differed markedly between the lakes. The BCC changed along the second axis in both lakes (Tuesday $r = 0.86$, $P < 0.05$; Peter $r = 0.93$, $P < 0.001$). This axis was correlated with temperature and Chl *a* (Table 2), i.e. variables changing over time in the lakes. PCA reduction of the quantitative t-RFLP data resulted in three principal components with eigenvalues greater than 1. The general pattern was comparable with that of NMDS analysis (data not shown). The first principal component (PC1) explained 40% of the variation in BCC, separated the two lakes and was correlated with water colour, DOC, TN, TP and pH (Table 2). PC2 explained another 14% and was correlated with temperature, Chl *a*, pH and TN (Table 2). PC3 explained 8% and was not correlated with any of the environmental variables. Thus, PCA of quantitative t-RFLP data gave similar results to NMDS of binary data.

To evaluate better how changes in BCC are related to environmental variables, we also analysed the two lakes separately. For Tuesday Lake this resulted in a two-dimensional NMDS (stress 0.11) and three principal components with eigenvalues greater than 1, explaining 32, 19 and 17% respectively of the variation in BCC. Overall, Dimension 1 and PC1 related to environmental factors in a similar fashion, being strongly correlated with temperature, Chl *a* and DOC (Table 2). Dimension 1 was also correlated with water colour. Moreover, Dimension 2 was associated with pH and PC3 with TN, whereas PC2 was not related to any measured environmental variables (data not shown). For Peter Lake, PCA resulted in four principal components explaining 40, 18, 13 and 9% of the variation in BCC, and a two-dimensional NMDS (stress 0.09). Dimension 1 and PC1 were both correlated with temperature, DOC and Chl *a* (Table 2). In addition, the PC1 was correlated with pH. Dimension 2 and the second, third and fourth components were not significantly related to any measured environmental parameters. Thus, in the two lakes, the seasonal succession correlated with similar environmental variables, because temperature, Chl *a* and DOC were significantly correlated to the first axis in all cases. Among these, temperature was the most strongly correlated with seasonal succession in both lakes.

In Tuesday and Peter lakes 40 and 44% of the t-RFs correlated with environmental variables, the majority of correlations being between t-RFs and Chl *a* and temperature in both lakes. Moreover, 34 and 27% of the t-RFs were correlated with BP in Tuesday and Peter lakes, respectively.

Table 2. Correlations between environmental variables and dimensions (Dim.) obtained from nonmetric multidimensional scaling and principal components (PC) from PC analysis of terminal restriction fragment length polymorphism (t-RFLP) patterns

	Both lakes together				Tuesday Lake		Peter Lake	
	Dim. 1	Dim. 2	PC1	PC2	Dim. 1	PC1	Dim. 1	PC1
pH	0.67***	0.43	-0.62***	-0.57**	-0.43	0.40	0.48	-0.59*
Water colour	-0.94***	-0.07	0.92***	0.09	0.69*	-0.51	0.49	-0.51
DOC	-0.85***	0.20	0.89***	-0.09	0.65*	-0.71**	0.66*	-0.79**
TP	0.78***	0.31	-0.66**	-0.36	-0.46	0.39	0.41	-0.20
TN	0.75***	0.47	-0.66**	-0.53*	-0.06	0.02	0.59	-0.43
Chl a	0.37	0.59*	-0.29	-0.72***	-0.83**	0.89**	0.63*	-0.68**
Temp.	-0.04	0.78***	0.24	-0.72***	0.87**	-0.93***	0.95***	-0.88***

Statistics in the first four columns refer to correlation analyses between environmental variables and bacterial community composition including both lakes, whereas the latter four represent correlations for the two lakes separately. The numbers are Pearson coefficients and the confidence intervals were established by bootstrapping. The degree of significance is indicated with asterisks:

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

DOC, dissolved organic carbon; TP, total phosphorus; TN, total nitrogen.

Table 3. Qualitative traits of the organic substrates from algae, birch, maple and fulvic acids, and the functional bacterial response in the experimental treatments

	Algae	Birch	Maple	Fulvic acids
DOC [†] :DOP [‡]	22	1626	1987	4793
DOC [†] :TDP [§]	17	305	238	145
$\epsilon_{250}/\epsilon_{440}$ [¶]	10.4	15.3	14.1	11.8
% DOC utilization	47	34	29	10
BP /DOC	0.18	0.10	0.10	0.03
BR ^{**} /DOC	0.30	0.27	0.20	0.07
BGE ^{††} (%)	38	29	32	28

[†]DOC, dissolved organic carbon.

[‡]DOP, dissolved organic phosphorus.

[§]TDP, total dissolved phosphorus (organic+inorganic).

[¶] $\epsilon_{250}/\epsilon_{440}$, molar absorption at 250 nm compared with that at 440 nm, indicating the amount of small molecules in relation to the amount of large molecules.

^{||}BP, bacterial production.

^{**}BR, bacterial respiration.

^{††}BGE, bacterial growth efficiency.

DOM-amended batch cultures

Aimed at doubling the DOC concentration in the water from Tuesday Lake, the additions of the various substrates gave an initial average concentration of DOC between 18 and 20.5 mg L⁻¹, the M treatments containing significantly more DOC than the other three treatments (Tukey's *post hoc* $P < 0.05$). The increase of P resulting from substrate additions varied substantially (Table 3). The A medium contained large amounts of P that was mainly organic, whereas the P content in the B, M and F media was lower and consisted of more than 80% of inorganic P. A further difference in quality among the substrates was that indicated by absorption coefficients (Table 3). As measured by $\epsilon_{250}/$

ϵ_{440} , the A and FA treatments had a higher proportion of large molecules than B and M. However, the ratio of $\epsilon_{250}/\epsilon_{440}$ did not relate in any discernable way to the measured response variables.

Bacterial growth was rapidly stimulated by the DOM amendments. BP in the B and M treatments reached maximum values after just 13 h and declined slightly after that. A and FA exhibited their highest BP after 25 h. Thereafter, BP in the F treatment remained stable whereas that of A decreased. Because DOC concentration was significantly higher in M than in the other treatments, differences in BP and BR were analysed based on numbers normalized for (divided by) DOC concentration. There were strong significant effects of the medium on the bacterial response (ANOVA: BP, BR and %DOC utilization $P < 0.001$ and BGE $P < 0.01$). PCA, with the functional parameters as variables, grouped the cultures into three distinct clusters (K-means clustering $P < 0.001$, Fig. 3a): A, F, and B together with M. BP/DOC was highest in A, intermediate in the leaf extracts and lowest in F (Tukey's *post hoc* $P < 0.01$). As for respiration, all treatments were significantly different, with the highest BR in A, followed by B, M and F. Because BP was more variable than BR, the % utilization of DOC largely followed the same pattern as BP, and was between 10 and 47%. BGE varied from 28% in FA to 38% in A (Table 3), and the A treatment was significantly higher than B and F (Tukey's *post hoc* $P < 0.05$).

Interestingly, ϵ_{440} decreased in all treatments during incubation, whereas ϵ_{250} increased. Thus large molecules were utilized in all treatments. That ϵ_{250} increased does not necessarily mean that smaller molecules were not utilized. A loss of small molecules may have been masked by the addition of new ones, either from bacterial exudation or cleavage of larger molecules.

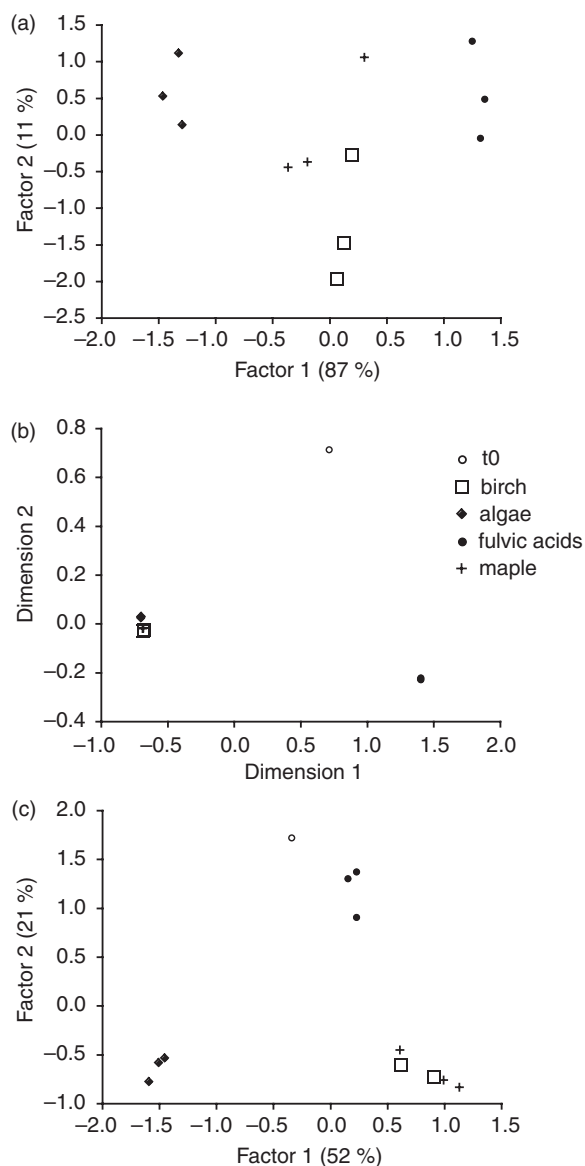


Fig. 3. Results from the batch culture experiment concerning function and community structure. (A) Principal component analysis (PCA) of functional parameters: bacterial production, respiration and growth efficiency and percentage utilization of dissolved organic carbon. (B) Non-metric multidimensional scaling analysis of community structure (presence/absence of terminal restriction fragment polymorphism (t-RFLP) peaks). The similarity within treatments was so large that replicates are not discernible in the diagram. Stress = 0.001. (C) PCA of community structure (area of t-RFLP peaks).

Upon digestion with the *Hae*III, *Hha*I and *Rsa*I restriction enzymes, 30, 60 and 139, different t-RFs were produced from the 11 samples (one replicate of B excluded). Altogether, 44% of t-RFLP fragments that appeared in the batches also appeared in either or both of the lakes. Only 3% of the peaks were found in all treatments (not necessarily in all replicates). The average proportion of shared peaks

between profiles from replicates was 53% (SD 7), and that of profiles from different treatments was 19% (SD 14). For the leaf extracts, the average within-treatment similarity (58%) was not significantly higher than the between-treatment similarity (46%; Tukey's *post hoc* $P > 0.05$). In all other cases, between-treatment differences were larger than within-treatment differences (Tukey's *post hoc* $P < 0.05$). Distances in the NMDS (Fig. 3b) ordination reflect the magnitude of dissimilarity in t-RFLP pattern well, as indicated by the goodness of fit (0.001) of the stress value for the ordination with two dimensions. PCA resulted in two principal components with eigenvalues greater than 1 that explained 52 and 21% respectively of the variation in BCC in the different treatments (Fig. 3c). NMDS and PCA analyses both confirmed that the bacterial community had become very different from that at t0. K-means clustering of NMDS and PCA also showed the presence of three clusters (ANOVA $P < 0.001$): A, F, and B together with M, i.e. similar to the functional clustering (Fig. 3a). However, ANOVA of cluster analysis of the NMDS pattern was also significant for 2 clusters only ($P < 0.001$): A, B and M together, and F separately.

Overall, the different batch cultures had similar fractions of t-RFs in common with Peter as with Tuesday Lake. Both lakes had the most t-RFs in common with the F treatment. Out of the t-RFs that covaried with Chl *a* in Peter Lake, 67% occurred in the batch cultures, 47% in the A treatment. Only 16% of those that correlated with Chl *a* in Tuesday Lake were found in any of the batch cultures.

Discussion

In this study the seasonal succession of the bacterioplankton was followed in two lakes differing widely in their source of organic matter for the bacterioplankton – the net-autotrophic Peter Lake, and the net-heterotrophic Tuesday Lake (Kritzberg *et al.*, in press). BCC differed markedly between the lakes. Analysis of environmental data in relation to t-RFLP data suggested that these differences were related to water colour, DOC, TP, TN and pH, i.e. variables that are connected to the relative loading of autochthonous and allochthonous C in the lakes, and to the fact that Peter Lake was manipulated with inorganic nutrients (Kritzberg *et al.*, in press; Bade, 2004). Because both lakes have relatively long residence times (approximately two years; Cole & Pace, 1998), the differences in BCC that we observe probably arise from differences in processes within these lakes, rather than from introductions of different allochthonous bacteria to the lakes (Lindström *et al.*, 2006). In addition, the lakes are situated too close to each other to be expected to differ because of confined distribution of bacterial populations (Yannarell & Triplett, 2005). Thus, altogether, although statistical relations cannot prove mechanistic relationships,

our data suggest that the differences in autochthonous vs. allochthonous loading of the lakes contributed to differences in BCC between the lakes.

Bacterial community composition changed continuously over the season in both lakes, and more so in manipulated Peter Lake than in Tuesday Lake. In both lakes the temporal change in BCC correlated with temperature, chlorophyll and DOC. The colinearity of temperature, chlorophyll and DOC in the lakes makes it impossible to determine whether each correlation is independent in its occurrence (Table 1). Temperature was the variable most strongly related to the change in BCC. Temperature can have a direct selective effect on BCC in lakes, because bacteria can exhibit different temperature optima (Hahn & Pöckl, 2005). In addition, temperature may influence BCC indirectly, for example by increasing planktonic metabolism and turnover of both organisms and nutrients. Several other field studies have also seen a statistical relationship between BCC and temperature (Van der Gucht *et al.*, 2001; Muylaert *et al.*, 2002; Yannarell *et al.*, 2003; Jardillier *et al.*, 2004; Yannarell & Triplett, 2004). Thus, temperature changes might have contributed to the observed shifts in BCC.

Besides temperature, concentrations of chlorophyll and DOC were also strongly correlated with BCC in the lakes. DOC increased in both lakes over time. There is evidence that the quantitative supply of C influences BCC at DOC concentrations below *c.* 6 mgL⁻¹ (Eiler *et al.*, 2003). In a survey of 6 Adirondack lakes, DOC concentrations also appeared to have an important influence on bacterial community structure (Méthé & Zehr, 1999). It can also be assumed that the increase in DOC concentration in Tuesday and Peter lakes was paralleled by a partial change in composition of DOC, which has a documented effect on the bacterial community (Van Hannen *et al.*, 1999; Findlay *et al.*, 2003; Pinhassi *et al.*, 2004; Langenheder *et al.*, 2005). It can be hypothesized that the DOC increase in Peter Lake was the result of increased primary production. However, DOC changed in a similar manner in Tuesday Lake, even though chlorophyll was decreasing over time. The simultaneous increase in DOC can not be connected to precipitation, which was very sparse during the period of study. However, to conclude, shifts in BCC in the lakes might have been caused by shifts in organic substrate quantity and quality (i.e. allochthonously or autochthonously produced).

In line with the correlation between chlorophyll and the seasonal succession of bacteria in Tuesday and Peter lakes, Crump and others (Crump *et al.*, 2003) found that, as the summer phytoplankton community in Toolik Lake, Alaska developed, the composition of the bacterial community changed, and they attributed this change to the production of fresh organic matter as a food source for bacteria. Similarly, BCC varied seasonally as a function of primary production in the Sep reservoir, France (Jardillier *et al.*,

2004), and in the Sacramento–San Joaquin River Delta the community structure changed along with the proportion of phytoplankton-derived DOM (Stepanaukas *et al.*, 2003). In addition, it has been shown that different taxonomic groups of bacteria relate differently to differing levels of primary productivity (Méthé & Zehr, 1999; Horner-Devine *et al.*, 2003). Thus, BCC covaries with phytoplankton, possibly as a result of changes in the quantity and quality of the DOC pool in the lakes.

In summary, the results from the field study in the two lakes indicate that the source, quantity and composition of organic substrate are among the factors shaping the composition of the bacterial communities in the lakes. In order to test this idea more explicitly, a batch culture experiment was set up. Bacteria from the humic Tuesday Lake were exposed to different sources of organic carbon, derived from algae (A), birch (B) and maple (M) leaves, and in addition fulvic acids (F). Even though there were differences between replicates, there was a clear effect of the substrate amendments on composition as well as function of the bacterial community. PCA and K-means clustering revealed that A, F and B, and M formed three distinct groups with regards to both BCC and function.

The bacterial community of the various treatments differed substantially from the initial inoculum taken from Tuesday Lake (t₀). Because incubations of the media without inoculum remained sterile, all bacterial taxa present in the batch cultures were also present in the inoculum, although not all of them were abundant enough to be detected by t-RFLP. t-RFLP is a powerful method for detecting differences in taxon composition and for assessing dynamics of BCC, but it does not allow a complete characterization of the bacterial community (Dahllöf, 2002). Others have pointed to the limitations of batch cultures, such as the possibility that they favour the growth of microbes different from the initial community as a result of, for example, reduced grazing pressure (Massana *et al.*, 2001). However, it has been argued that the populations selected during bottle incubations are the ones actively growing but maintained at low abundance *in situ* by grazing (Suzuki, 1999). This idea is supported by the finding that bacteria that are rare *in situ*, but are easily cultivated, and thus grow fast in culture, are sensitive to flagellate grazing (Beardsley *et al.*, 2003). Thus, although changes in BCC in lakes over time provide some information about the activity of individual populations, the identity of populations responsible for bacterial production may be better studied in filtered batch cultures in which the number of flagellates are reduced, and, thus, predator-sensitive but active bacteria can be detected.

There were some links between the bacterial dynamics in the lakes and in the batch cultures. In total, 44% of the t-RFs found at termination of the batch cultures were found in at

least one of the lakes. Many of the t-RFs that covaried with chlorophyll in Peter Lake were found in the batch cultures (67%) and especially so in the A treatment, suggesting that these particular bacterial taxa are favoured by high-quality DOM. On the other hand, only a few of the t-RFs that correlated with chlorophyll in Tuesday Lake were found in the batch cultures (16%), which may indicate that it was not primarily the input of phytoplankton C that regulated BCC in this lake, but perhaps temperature or DOC. This is in agreement with the finding that bacteria in Tuesday Lake derive only 22% of bacterial production from autochthonous C (Kritzberg *et al.*, in press). Both lakes shared most t-RFs with the fulvic acid treatment, possibly because this treatment was the one least enriched with regards to nutrients. Additions of inorganic P have been shown to generate reproducible, although small, changes in BCC (Lindström *et al.*, 2004), and additions of N and P alone have been reported to yield larger shifts in BCC than NP + DOM (Fisher *et al.*, 2000). However, although inorganic nutrients might have played a role, the results from the batch culture experiments support the results from the field study; that is, the organic substrate source might have contributed to the shaping of the bacterial communities in the lakes.

In this experiment, the different bacterial communities had distinct functions (Fig. 3): DOC utilization, bacterial production, respiration and growth efficiency were highest on the A substrate, intermediate on B and M, and lowest on F. Findlay and others (Findlay *et al.*, 2003) found changes in community metabolism along with shifts in community composition after adding various sources of DOM to hyporheic bacterial communities. Similarly, a shift in both the community composition and enzyme activities of bacteria paralleled the progression of a mesocosm diatom bloom, as reported by Riemann *et al.* (2000). Thus, together these separate studies suggest that there may be a connection between community structure and organic carbon utilization. There is, however, conflicting evidence, indicating that the coupling between composition and function may be weak. In a full-factorial switch design experiment, investigating the influence of growth medium vs. inoculum on BCC and function, Langenheder and others (Langenheder *et al.*, 2005) found that functional parameters related to C utilization were influenced only by the medium, and not by community structure. In a different batch culture experiment, in which replicates of an identical medium were inoculated with bacteria from 8 different sites (Langenheder *et al.*, 2006), bacterial production, respiration and growth efficiency were largely independent of BCC, whereas ectoenzyme activity and the potential to utilize benzoic acid differed at least partly between different bacterial communities. Hence, while our study adds to the evidence supporting a coupling between community structure and function, it appears that the strength of such a coupling is variable.

One major difference between the two sets of experiments discussed above is the degree of manipulation. While the present study and that by Findlay *et al.* (2003), in which substrate additions included glucose, bovine serum albumin and leaf leachates, represent rather substantial changes in the DOM source, the studies by Langenheder *et al.* (2005, 2006) involve less dramatic manipulations using different natural waters. Hence, one explanation of the discrepant results from the two sets of experiments could be that a substantial change in the DOM source is needed to trigger a concomitant change in community composition and function. If only minor changes in substrate quantity and quality occur, this factor might have little influence on BCC and function, while other factors, such as the source community of the inocula (Langenheder *et al.*, 2005, 2006), influence BCC to a larger extent. Owing to a high degree of functional redundancy, communities differing in BCC can exhibit similar functions under similar conditions, and thus the coupling between structure and function appears to be weaker. It can also be speculated that large manipulations result in structural changes on a higher phylogenetic level, and that functional redundancy is more common at the 'species'/ribotype level rather than between major bacterial groups. In support of this are results showing that major bacterial groupings (Alpha, Beta and Gammaproteobacteria and *Bacteroidetes*) show differences in enzyme activities and consumption of organic compounds as well as in preference for nutrient concentrations (Cottrell & Kirchman, 2000; Pinhassi & Berman, 2003; Kirchman *et al.*, 2004). One other possible explanation of the fact that different studies show a variable degree of coupling between BCC and function is that specialized functions, such as enzyme activities, may have another connection to BCC than more broad-scale functions such as respiration and growth rate, which might be more taxa-independent (Langenheder *et al.*, 2006).

To summarize, we found that source, concentration and composition of organic matter might be among the factors influencing BCC (both among lakes and on a seasonal scale) as well as general bacterial functions such as production, respiration and growth yield.

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References

- Cottrell MT & Kirchman DL (2000) Natural assemblages of marine proteobacteria and member of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular weight dissolved organic matter. *Appl Environ Microbiol* **66**: 1692–1697.
- Bade DL (2004) Ecosystem carbon cycles: whole-lake fluxes estimated with multiple isotopes. PhD thesis, University of Wisconsin, Madison, WI.
- Beardsley C, Perntaler J, Wosniok W & Amann R (2003) Are readily culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? *Appl Environ Microbiol* **69**: 2624–2630.
- Carpenter SR, Kitchell JF, Cottingham KL, Schindler DE, Christensen DL, Post DM & Voichick N (1996) Chlorophyll variability, nutrient input and grazing: evidence from whole-lake experiments. *Ecology* **77**: 725–735.
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Austr J Ecol* **18**: 117–143.
- Coffin RB & Cifuentes LA (1999) Stable isotope analysis of carbon cycling in the Perdido Estuary, Florida. *Estuaries* **22**: 917–926.
- Cole JJ & Pace ML (1998) Hydrologic variability of small, Northern Michigan lakes measured by the addition of tracers. *Ecosystems* **1**: 310–320.
- Cole JJ, Caraco NF, Kling GW & Kratz TK (1994) Carbon dioxide supersaturation in the surface waters of lakes. *Science* **265**: 1568–1570.
- Covert JS & Moran MA (2001) Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. *Aquat Microb Ecol* **25**: 127–139.
- Crump BC, Kling GW, Bahr M & Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter sources. *Appl Environ Microbiol* **69**: 2253–2268.
- Dahllöf I (2002) Molecular community analysis of microbial diversity. *Curr Opin Biotechnol* **13**: 213–217.
- D'Elia CF, Stuedler PA & Corwin N (1977) Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol Oceanogr* **22**: 760–764.
- Egert M & Friedrich MW (2003) Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. *Appl Environ Microbiol* **69**: 2555–2562.
- Eiler A, Langenheder S, Bertilsson S & Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Appl Environ Microbiol* **69**: 3701–3709.
- Findlay SEG, Sinsabaugh RL, Sobczak WV & Hoostal M (2003) Metabolic and structural responses of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnol Oceanogr* **48**: 1608–1617.
- Fisher MM, Klug JL, Lauster G, Newton M & Triplett EW (2000) Effects of resources and trophic interactions on freshwater bacterioplankton diversity. *Microb Ecol* **40**: 125–138.
- del Giorgio PA, Cole JJ & Cimperlis A (1997) Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385**: 148–151.
- Hahn MW & Pöckl M (2005) Ecotypes of planktonic Actinobacteria with identical 16S rRNA genes adapted to thermal niches in temperate, subtropical and tropical freshwater habitats. *Appl Environ Microbiol* **71**: 766–773.
- Horner-Devine MC, Leibold MA, Smith VA & Bohannan BJM (2003) Bacterial diversity patterns along a gradient of primary productivity. *Ecol Lett* **6**: 613–622.
- Jardillier L, Basset M, Domaizon I, Belan A, Amblard C, Richardot M & Debroas D (2004) Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir. *Aquat Microb Ecol* **35**: 259–273.
- Karlsson J, Jansson M & Jonsson A (2002) Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. *Ecology* **83**: 2902–2910.
- Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *Handbook of Methods in Aquatic Microbial Ecology*, (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds), pp. 509–512 Lewis Publishers, Boca Raton, FL.
- Kirchman DL, Dittel AI, Findlay SEG & Fischer D (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquat Microb Ecol* **35**: 243–257.
- Kritzberg ES, Cole JJ, Pace ML, Granéli W & Bade D (2004) Autochthonous versus allochthonous carbon sources to bacteria: results from whole-lake ¹³C addition experiments. *Limnol Oceanogr* **49**: 588–596.
- Kritzberg ES, Cole JJ, Pace ML & Granéli W (2005) Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs? *Aquat Microb Ecol* **38**: 103–111.
- Kritzberg ES, Cole JJ, Pace ML & Granéli W (in press) Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: Results from whole-lake ¹³C addition experiments. *Ecosystems*, in press.
- Lane DL, Pace B, Olsen GL, Stahl DA, Sogin ML & Pace NR (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. *PNAS* **82**: 6955–6959.
- Langenheder S, Lindström ES & Tranvik LJ (2005) Weak coupling between community composition and functioning of aquatic bacteria. *Limnol Oceanogr* **50**: 957–967.
- Langenheder S, Lindström ES & Tranvik LJ (2006) Structure and function of bacterial communities emerging from different

- sources under identical conditions. A test of the ubiquity of aquatic bacteria. *Appl Environ Microbiol* **72**: 212–220.
- Lindström ES & Bergström AK (2004) Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time. *Limnol Oceanogr* **49**: 125–136.
- Lindström ES, Forslund M, Algesten G & Bergström A-K (2006) External control of bacterial community structure in lakes. *Limnol Oceanogr* **51**: 339–342.
- Lindström ES, Vrede K & Leskinen E (2004) Response of a member of the Verrucomicrobia, among the dominating bacteria in a hypolimnion, to increased phosphorus availability. *J Plank Res* **26**: 241–246.
- Magurran AE (1988) *Ecological Diversity and its Measurement*. Princeton University Press, Princeton, NJ.
- Massana R, Pedrós-Alió C, Casamayor EO & Gasol JM (2001) Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnol Oceanogr* **46**: 1181–1188.
- Méthé BA & Zehr JP (1999) Diversity of bacterial communities in Adirondack lakes: do species assemblages reflect lake water chemistry? *Hydrobiologia* **401**: 77–96.
- Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M & Vyverman W (2002) Relationship between bacterial community compositions and bottom-up versus top-down variables in four eutrophic shallow lakes. *Appl Environ Microbiol* **68**: 4740–4750.
- Øvreås L, Forney L, Daae FL & Torsvik V (1997) Distribution of bacterioplankton in meromictic lake Sælenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S RNA. *Appl Environ Microbiol* **63**: 3367–3373.
- Pinhassi J & Berman T (2003) Differential growth response of colony-forming α - and γ -proteobacteria in dilution culture and nutrient addition experiments from Lake Kinnaret (Israel), the Eastern Mediterranean Sea, and the Gulf of Eilat. *Appl Environ Microbiol* **69**: 199–211.
- Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol Ò, Malits A & Marrasé AM (2004) Changes in bacterioplankton compositions under different phytoplankton regimens. *Appl Environ Microbiol* **70**: 6753–6766.
- Riemann L, Steward GF & Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66**: 578–587.
- Smith DC & Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in sea water using ^3H -leucine. *Mar Microb Food Webs* **6**: 107–109.
- Stanton MP (1973) A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in freshwater and carbonates in sediments. *J Fish Res Board Can* **30**: 1441–1445.
- Stepanuskas R, Moran MA, Bergamaschi BA & Hollibaugh JT (2003) Covariance of bacterioplankton composition and environmental variables in a temperate delta system. *Aquat Microb Ecol* **31**: 85–98.
- Strome DJ & Miller MC (1978) Photolytic changes in dissolved humic substances. *Verh Int Verein Limnol* **20**: 1248–1254.
- Suzuki MT (1999) Effect of protistan bacterivory on coastal bacterioplankton diversity. *Aquat Microb Ecol* **20**: 261–272.
- Van der Gucht K, Sabbe K, De Meester L, Vloemans N, Zwart G, Gills M & Vyverman W (2001) Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. *Environ Microbiol* **3**: 680–690.
- Van Hannen EJ, Mooij W, Van Agterveld MP, Gons HJ & Laanbroek HJ (1999) Detritus-dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **65**: 2478–2484.
- Vergin KL, Urbach E, Stein JL, DeLong EF, Lanoil BD & Giovanni SJ (1998) Screening a fosmid library of marine environmental genomic DNA fragments reveals four clones related to members of the order Planctomycetales. *Appl Environ Microbiol* **64**: 3075–3078.
- Weinbauer MG & Höfle MG (1999) Distribution and life strategies of two bacterial populations in a eutrophic lake. *Appl Environ Microbiol* **64**: 3776–3783.
- Yannarell AC & Triplett EW (2004) Within- and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. *Appl Environ Microbiol* **70**: 214–223.
- Yannarell AC, Kent AD, Lauster GH, Kratz TK & Triplett EW (2003) Temporal patterns in bacterial communities in three temperate lakes of different trophic status. *Microb Ecol* **46**: 391–405.
- Yannarell AC & Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. *Appl Environ Microbiol* **71**: 227–239.