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The interplay between bacterial community composition and the environment determining function of inland water bacteria

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Abstract

We hypothesized that habitats differing in water flow regime would differ in bacterial function either because of differences in the local environment, in bacterial community composition (BCC), or in the mechanism shaping BCC (community assembly). In 20 lakes and 17 inlet streams BCC was analyzed by terminal restriction fragment length polymorphism of the gene coding for 16S ribosomal RNA, and bacterial function was estimated as bacterial production rate (BP, measured as leucine incorporation) per content of dissolved organic carbon (DOC) (BP : DOC). BCC in both lakes and streams appeared to be shaped by local environmental forces (i.e., species sorting according to metacommunity theory), but not by massive introduction of cells from the drainage area (mass effect). BP : DOC was lower in streams than in lakes, which appeared to be both because of differences in BCC and environment between lakes and streams, independent of each other. We found no support for an effect of water flow regime in itself (i.e., cell dispersal rate) causing the lower functionality of the streams. In streams, BP : DOC was correlated to both BCC and environment, independent of each other, while in lakes function could not be explained by either BCC or environment. The greater environmental variability among our streams than among our lakes may be the cause for the stronger BCC-function coupling in our streams, since smaller environmental variation among our lakes would allow a greater functional redundancy.

In aquatic pelagic ecosystems, a pivotal role of bacteria is in the cycling of carbon. Bacteria serve as a link between dissolved organic matter and higher trophic levels through the ''microbial loop'' (Azam et al. 1983) and the ''reversed microbial loop'' (Jansson et al. 1996). Through the use of organic matter that would otherwise be unavailable to the aquatic community, bacteria contribute both to the planktonic biomass production as well as to carbon dioxide emission. Both bacterial communities and dissolved organic matter pools are complex, and bacteria differ in their ability to process organic matter of different composition (Martinez et al. 1996; Cottrell and Kirchman 2000), and, thus, a question of profound importance is whether the structure of the bacterial community determines how well the organic matter can be used.

The role of biodiversity for ecosystem functioning has emerged as a major field within ecological research (Loreau et al. 2001; Gamfeldt and Hillebrand 2008). Despite the great diversity of microorganisms, their significant role in ecosystems, and their potential as model organisms (Jessup et al. 2004; Prosser et al. 2007), most studies of biodiversity-functioning relationships have been conducted on larger organisms. Recently, studies investigating the importance of the bacterial community composition (BCC) for important ecosystem functions have received considerable attention. However, focusing on inland water bacteria, the results have not been entirely consistent. While some have seen a tight coupling between BCC and carbon processing functions in bacteria (Langenheder et al. 2006; Bertilsson et al. 2007), others have found the connection to be fairly loose, and that function depends more on the characteristics of the environment (Langenheder et al. 2005; Comte and Del Giorgio 2009). Thus, a particular bacterium may function differently under different circumstances, or different bacteria can act similarly under similar conditions (functional redundancy). However, the environment can also affect function indirectly by shaping BCC, which in turn can be of importance for function (Kirchman et al. 2004; Judd et al. 2006). A tight interplay between BCC, function, and environment makes study design for explicit testing of the importance of community structure for function very challenging (Reed and Martiny 2007) and can be one reason why the diversity-function relationship does not come across as apparent.

The discrepant results may also depend on the functional variable that is tested (Langenheder et al. 2006; Gamfeldt et al. 2008), i.e., some functions require a high degree of specialization while others can be performed by many different organisms, giving different relationships between BCC and function. Another possibility is that the function may depend on how the local community was formed. For instance, it was recently shown experimentally that bacterial growth may be maximized at intermediate dispersal rate from the regional species pool (metacommunity) (Venail et al. 2008). Likewise it has been suggested that the forces shaping BCC may differ between high dispersal communities and such experiencing a moderate dispersal from the lake's metacommunity (Logue and Lindström 2008), i.e., that the former type of communities are shaped by the massive introduction of cells and the latter primarily by local contemporary habitat conditions, similar to the mass effect and the species sorting scenario, respectively, according to metacommunity theory (Leibold et al. 2004). An interesting aspect to investigate is therefore whether different dispersal regimes in nature give rise to differences in the relative importance of forces shaping

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BCC, and if that has consequences for bacterial function. Different dispersal regimes of planktonic communities require differences in water flow rates to their respective habitats. Differences in water retention time of inland waters lead to differences in organic matter quality (Sobek et al. 2003), which may also influence function directly or indirectly via changes in BCC as argued above.

The aim of this study was to investigate whether habitats differing in water flow regime also differ in an important bacterial carbon processing function, because of differences in either the environment, BCC, or mechanisms shaping BCC between the two types of habitats. In a field study including 20 lakes and 17 streams, we tested the hypothesis that aquatic bacterial communities that differ in water flow regime differ in (1) in the functionality of the bacterial community; (2) the coupling between BCC and function; and (3) the relative importance of mass effect and species sorting shaping local BCC. For this purpose we defined bacterial functionality as the amount of bacterial biomass produced per unit of dissolved organic carbon (BP : DOC), i.e., the ability of the bacterial community to use the resources present.

Methods

Study site and sampling—20 lakes and 17 associated inlets were sampled in July 2007. The sampled lakes are all less than 100 km apart in the region of Småland, Sweden (ca 57°N–15°E). The area is dominated by forest, and the lakes are oligotrophic (dissolved phosphorus 1–14 μg L^{-1}) with low alkalinity (0–20 μmol L^{-1}). The sampled lakes vary in size from 0.3 to 3.3 km^2 and have theoretical retention times in between 40 and 2800 d as calculated from the specific runoff at the day of the sampling (obtained from the Swedish Meteorological and Hydrological Institute). Only two pairs of lakes in the dataset are interconnected via streams. The 17 sampled streams were mostly small first-order streams, but a few second-order streams were included, as was one third-order stream. The streams are also in most cases of an oligotrophic character (dissolved phosphorus 3–68 μg L^{-1}). The theoretical water retention time of the streams was estimated using the specific runoff at the day of the sampling, stream length, and the drainage area of the stream as defined by height curves in ArcView (Environmental Systems Research Institute—Geographic Information System and mapping software) using Swedish Reference Frame 1999 (SWEREF99 maps). In absence of data on stream volume, which is required to calculate retention time, the stream length was multiplied with a deliberate overestimate of the average cross section area of the streams. Hence the achieved retention times are overestimates. When assuming a cross section area of 5 m^2 the retention time of the streams varies between 2 and 55 days.

The lakes were sampled over the deepest point. Water chemistry parameters were determined from an integrated sample of the epilimnion collected with a Plexiglas tube sampler. Samples for bacterial production and community composition were taken from an integrated sample of the upper 2 m using a 2-m Plexiglas tube. The stream was sampled approximately 20 m upstream from the lake, and samples for all analyses were collected just below the surface.

Analytical methods—pH was measured using a 206 pH3 pH meter (Testo). Dissolved nitrogen (DN), phosphorus (DP), dissolved organic carbon (DOC), and absorbance were analyzed on GF/F filtered water (Whatman). Inorganic nutrients were analyzed using a FIASTAR TM 5000 (Foss) following International Organization for Standardization (ISO) protocols ISO 11732, ISO 13395, and ISO/FDIS 15681-1. Organic phosphorus was analyzed using inductively coupled plasma mass spectroscopy (ICP MS) on Elan 6000 (Perkin Elmer). Organic carbon and nitrogen analyses were performed using a total organic carbon analyzer with combustion catalytic oxidation, nondispersive infrared method (TOC-VCPH) with a TNM-1 (Schimadzu). Ultraviolet and visible absorbance was measured at room temperature on a Coulter DU800 spectrophotometer (Beckman) with a 1-cm cuvette at 280 and 440 nm (Abs_{280} and Abs_{440}). The specific absorbance (SA_{280} and SA_{440}) was determined by dividing Abs_{280} and Abs_{440} by the dissolved organic carbon (DOC) concentration. Spectral slope was obtained using a linear regression of the natural logarithm of the absorption coefficient vs. wavelength in the range 280–500 nm (Zhang and Qin 2007).

Bacterial production was estimated by measuring incorporation of tritiated leucine (Amersham TRK 510, specific activity 23.3 Ci $mmol^{-1}$, final concentration 100 nmol L^{-1}). Incubations were performed during 60 min in triplicate 2-mL Eppendorf tubes plus one TCA-killed (trichloroacetic acid) control and were terminated by the addition of TCA (5% final concentration). The water temperature in lakes and inlets varied between 16°C and 20°C, and the incubations were performed at 18°C. The bacteria were concentrated to a pellet by centrifugation at 14,000 × *g* for 10 min. The pellet was washed with 5% TCA and 80% ethanol. Finally, 0.5 mL of scintillation cocktail (Ecoscint A, National Diagnostics) was added and ^3H activity measured on a LS 6500 Scintillation counter. Leucine incorporation rates were transformed into bacterial C production rates using a conversion factor of 3.1 kg C mol^{-1} incorporated leucine, following Smith and Azam (1992).

BCC—BCC was analyzed by terminal restriction fragment length polymorphism (tRFLP) (Osborn et al. 2000; Eiler and Bertilsson 2004). Water samples of 2 mL were transferred to sterilized Eppendorf tubes and centrifuged at 14,000 × *g* for 30 min. The supernatant was discarded, and the procedure repeated, so that finally each Eppendorf tube contained a bacterial pellet from 4 mL of sample water. The pellets were then frozen and stored at −80°C. Deoxyribonucleic acid (DNA) extraction of the pellet was carried out using the 5 Prime kit (VWR) (ArchivePure DNA yeast and Gram-positive bacteria kits) following a slightly modified protocol such as: the pellets were dissolved in 100 μL lysis-buffer (20 mmol L^{-1} Tris·Cl, pH 8.0, 2 mmol L^{-1} sodium ethylenediamineteraacetic acid (EDTA), 1.2% Triton® X-100) containing 20 mg L^{-1}

lysozyme (lysozyme is added immediately before use) and incubated at 37°C for 30 min. After extraction according to the 5 Prime protocol, DNA was precipitated with 1200 μL of 100% ethanol, 40 μL sodium acetate (3 mol L^{-1}, pH 5.2), and 0.8 μL glycogen at −80°C overnight. The DNA pellet was subsequently washed with 1000 μL of 70% ethanol and allowed to dry and elute with 30 μL of rehydrating solution from the kit.

Bacterial 16S ribosomal ribonucleic acid (16S rRNA) genes were amplified by polymerase chain reaction (PCR) from the extracted genomic DNA (diluted to 5 or 10 times) with the bacterial forward primer 27f (5′-AGRGTTT-GATCMTGGCTCAG-3′) (Vergin et al. 1998) and the universal reverse primer 519r (5′-GWATTACCGCG-GCKGCTG-3′) (Lane et al. 1985). The forward primer was labeled with hexachlorofluoroscein at the 5′ end (MWG Biotech) to enable fluorescence detection of terminal restriction fragments (tRFs) later. Each DNA extract was amplified in triplicates in 20 μL of PCR reaction solution in a Chromo 4 four-color real-time PCR system (Bio-Rad). Each 20 μL of reaction solution included 1 μL of the 5 or 10 times diluted DNA, 4 μL of 5× phusion HF buffer (Finnzymes), 200 nmol L^{-1} of each primer, 200 μmol L^{-1} of each dNTP (deoxyadenosine triphosphate, deoxyguanosine triphosphate, thymidine triphosphate, and deoxycytidine triphosphate), 0.02 units μL^{-1} of phusion enzyme (Finnzymes), and 0.1 μg μL^{-1} of T4 gene 32 protein (New England Biolabs). The reaction condition was an initial denaturation at 98°C for 30 s followed by 32 cycles of 10 s at 98°C, 30 s at 50°C and 30 s at 72°C, with a final extension at 72°C for 7 min.

The three replicates from the PCR amplification above were pooled together and purified by using QIAquick PCR purification kit (Qiagen). The purified products were then electrophoretically separated on a 1% agarose gel including ethidium bromide and quantified by comparing the bands in the gel against a Low DNA Mass™ Ladder (Invitrogen) using Gel-Pro Analyser™ version 3.1 (Media Cybernetics). For each purified PCR product, two replicates of enzyme restrictions were done. For each enzyme restriction, an aliquot of 40 ng of PCR product was added in a 10 μL reaction volume with 4 units of restriction endonuclease. Digestions were carried out separately with two four-cutter restriction enzymes *HaeIII* (Invitrogen) and *HinfI* (Invitrogen) in 96 wells for at least 16 h at 37°C. The enzyme activity was stopped by incubating at 72°C for 20 min.

Fluorescently labeled tRFs were separated by size in an ABI 3700 96-capillary sequencer running in GeneScan mode (Applied Biosystems) at Uppsala Genome Centre at Uppsala University, Uppsala, Sweden. tRFLP electropherograms were tabulated in GeneMarker 1.6 (SoftGenetics). A baseline threshold of 100 fluorescence units was used to determine "true peaks" from background noise. Peaks (tRFs) with length less than 50 base pairs (bp) and greater than 460 bp were eliminated from the analysis. Peaks less than 0.5 bases apart from each other were merged. Raw peak areas were relativized to total peak area of each run and all peaks smaller than 0.5% were removed to account for uncontrolled differences in the quantity of DNA between samples.

The average relative peak area of the restriction digest duplicates was used for further statistical analysis. For one sample, one duplicate for *HinfI* was missing and, thus, only the one run was used. Finally the data from the two restriction enzyme digests were put together in one data set.

Statistics—Differences between lakes and streams regarding environmental and functional variables (Table 1) were analyzed by Mann–Whitney *U*-tests using untransformed data.

Bray–Curtis distances (Legendre and Legendre 1998) between pairs of samples were calculated from untransformed relative tRFLP peak areas. The resulting distance matrix was used in a nonmetric multidimensional scaling analysis (MDS). The MDS was run with a random initial configuration and 500 iterations and 5 repetitions. The number of axes was chosen to obtain a Kruskal's stress < 0.14, which led to four axes for all data, four axes for lakes analyzed separately, and four axes for streams analyzed separately. The axes were termed MDS 1–4 and were used in subsequent statistical analyses as described below. The environmental data (E data i.e., DOC, DN, DP, pH, Abs_{280}, Abs_{440}, and spectral slope) were log-transformed (except for pH) and the values subsequently transformed to *z*-scores as

$$z_i = \frac{y_i - \bar{y}}{s_y}$$

where s_y is the standard deviation and $\bar{y}$ is the mean of a variable (Legendre and Legendre 1998). The transformed E data were analyzed by principal component analysis (PCA). The PCA were run as Pearson's correlations. In three streams spectral slope data were missing, and estimates of these values were calculated from means. The first two PC axes represented 88%, 85%, and 84% of the variation within the E data sets for all data, lakes only, and streams only, respectively. The first PC axis was termed PC1, the second PC2, and so on.

In order to test for covariations between bacterial function and BCC on one hand and function and environment on the other, the resulting MDS sample scores and the sample scores from all PC axes (seven per data set) were correlated to BP : DOC by Spearman rank correlation analyses. In cases when both MDS axes and PC axes were significantly correlated to BP : DOC Spearman rank partial correlation analyses were run to disentangle the single contribution by BCC and environment, respectively, independent of each other.

To evaluate the possible importance of habitat (lake or stream) in itself, i.e., independent of BCC or environment, analyses of covariance (ANCOVA) were run with BP : DOC (arcsin square root transformed) as the dependent variable, and a dummy variable (lake or stream) as well as MDS or PC axes as independent variables. The MDS and PC axes being included in this analysis were only those that were significant in the partial correlation analysis. Since not all variables were normally distributed, the analysis was run with original data as well as with ranked data.

Table 1. Comparison between lakes and streams. Mean values for lakes and streams, respectively, standard deviation (in brackets), and results from Mann–Whitney *U*-tests are shown. MDS axis all = sample scores obtained in MDS analysis of lakes and streams together. ns = no significant difference between lakes and streams.

	Lakes	Streams	*p* value
BP : DOC (10^{-3} d^{-1})	0.40 (0.22)	0.17 (0.17)	<0.001
BP (μg C L^{-1} d^{-1})	4.7 (3.0)	5.0 (5.0)	ns
E variables			
pH	6.53 (2.6×10^{-6})*	5.03 (6.0×10^{-5})	<0.001
DOC (mg L^{-1})	12.4 (6.59)	35.4 (18.4)	<0.0001
DN (μg L^{-1})	462 (196)	1176 (588)	<0.0001
DP (μg L^{-1})	4.0 (2.7)	18.6 (18.0)	<0.0001
SA_{440} (m^2 g^{-1})	0.29 (0.07)	0.43 (0.16)	0.001
SA_{280} (m^2 g^{-1})	3.4 (0.7)	4.8 (1.7)	0.001
Abs_{440} (cm^{-1})	0.037 (0.025)	0.168 (0.127)	<0.0001
Abs_{280} (cm^{-1})	0.442 (0.29)	1.940 (1.561)	<0.0001
Spectral slope (μm^{-1})	16.3 (1.07)	15.2† (1.08)	<0.01
Retention time (d)	350	9‡	<0.0001
BCC			
MDS all axis 1			<0.0001
MDS all axis 2			0.009
MDS all axis 3			0.02
MDS all axis 4			<0.0001

* Recalculated as $[H^+]$.
† This average is based on values of spectral slope from 14 streams only due to missing values.
‡ This is a calculated overestimate based on an assumed average cross section area of 5 m^2 (*see* Methods). Moreover the number includes only the values of 16 streams since one of the streams is fed by a lake.

Since the calculation of diversity indices from tRFLP data has been questioned (Blackwood et al. 2007) we chose to relate our functional variable only to BCC and not to other aspects of diversity, such as richness.

The potential importance of massive cell dispersal for BCC in the lakes (i.e., mass effect) was analyzed by correlating (Spearman rank correlation analysis) the similarity between lakes and inlets (Bray–Curtis similarities) to the water retention time of the lakes at the day of sampling.

Mann–Whitney *U*-tests, correlation analyses, ANCOVA, MDS, and PCA were run in XLSTAT 2009.1.01 for Macintosh. Partial correlations were run in Tanagra 1.4.31 (Rakotomalala 2005).

In order to analyze which factors covaried with BCC, redundancy analysis (RDA) was performed. First a detrended correspondence analysis (DCA) of the tRFLP data was performed giving a length of the first gradient of 2.14–2.29, and therefore a linear model was assumed and RDA was chosen (ter Braak 1995). tRFLP data (relative peak areas) were square root transformed, which should be comparable to Hellinger-transformation, a transformation method shown to circumvent the problems associated with linear models such as RDA (Legendre and Gallagher 2001).

The full data set of E variables tested was the same as in the PCA above and transformed in the same way. The variables within the E data set included in the models were chosen according to the procedure suggested by Blanchet et al. (2008).

Spatial predictors describing the geographic distribution of lakes (S variables) were constructed using principal coordinates of neighbor matrices (PCNM), as described by Griffith and Peres-Neto (2006). Starting with xy geographic coordinates of the lakes and the streams, Euclidean distance matrices (one for lakes and one for streams) were constructed, from which the eigenvectors with associated positive eigenvalues were extracted and used as spatial descriptors (5 for lakes and 4 for streams). Spatial predictors were calculated using the software SAM 3.0 (Rangel et al. 2006).

When lakes and streams were analyzed together, a dummy variable (lake or stream) was included. In order to analyze the relative importance of the E variables and the dummy variable, variance partitioning (Borcard et al. 1992) was performed. The purpose of this operation was to investigate whether there were unique lake and stream bacteria, independent of the difference in environment between the two types of habitats.

RDA and DCA were run using the software Canoco 4.5 (Biometris). RDA was run, focusing scaling on interspecies correlations, and centering by species. Significance testing of RDA models was done with 999 Monte Carlo permutations under reduced model, all canonical axes. The variance in community structure explained was entered in Table 2 calculated as unbiased estimates of variation components (Beisner et al. 2006; Peres-Neto et al. 2006).

Results

BCC in relation to environmental variables—BCC differed between lakes and streams to some extent (Fig. 1; Table 1). 276 different tRFLP peaks were identified, 205 in lakes and 209 in streams. None of the peaks was detected in all samples. Exactly 50% of the peaks were detected in both

Table 2. Results from RDA. The table shows the variance explained (unbiased estimates in %) by spatial predictors alone (S), environmental variables alone (E), the lake–stream dichotomy (L–S), and the shared contribution by the lake–stream dichotomy and environment (L–S + E). nt = not tested.

	S	E	L–S	L–S + E	Unexplained variance	Most important E variables
Lakes	0	23	nt	nt	77	pH, DP
Streams	0	17	nt	nt	83	pH, spectral slope
All data	nt	10	2	23	65	pH, DN, spectral slope

lakes and streams; 25.7% only in streams; and 24.3% only in lakes.

Out of the variation in BCC, 35% could be explained by environmental variables or the lake–stream dichotomy, i.e., the fact that a sample was collected in a lake or in a stream (RDA; Table 2). The greatest fraction was explained by the shared contribution by the lake–stream dichotomy and the environment (23%). The environment alone explained 10%, the most important variable being pH, but several other variables were also significant. However, 2% of the variation in BCC could be explained by the lake–stream dichotomy alone, i.e., lakes and streams appeared to harbor different bacterial populations also independent of differences in the environment.

RDA of the lake data showed no significant contribution by spatial predictors ($p = 0.99$), while environmental variables could explain 23% of the variation in BCC. The most important E variable was pH, but DP was also significant. There was no significant correlation between the similarity in BCC between lakes and inlets and the water retention time of the lakes at the day of sampling ($p > 0.05$, data not shown). Only two lake–stream pairs had a Bray–Curtis similarity > 0.5.

RDA of the stream data showed no significant contribution by spatial predictors ($p = 0.19$), while environmental variables could explain 17% of the variation in BCC. The most important E variable was again pH, but also spectral slope was significant.

Function in relation to BCC and the environment—In general, environmental variables as well as BP varied more among the streams than among the lakes, but there were also significant differences between lakes and streams (Table 1; Fig. 2). Bacterial production (BP) did not differ between lakes and streams, while BP : DOC was significantly higher in lakes than in streams as shown by Mann–Whitney *U*-test (Table 1; Fig. 3). The streams further had higher contents of dissolved carbon, nitrogen, and phos-

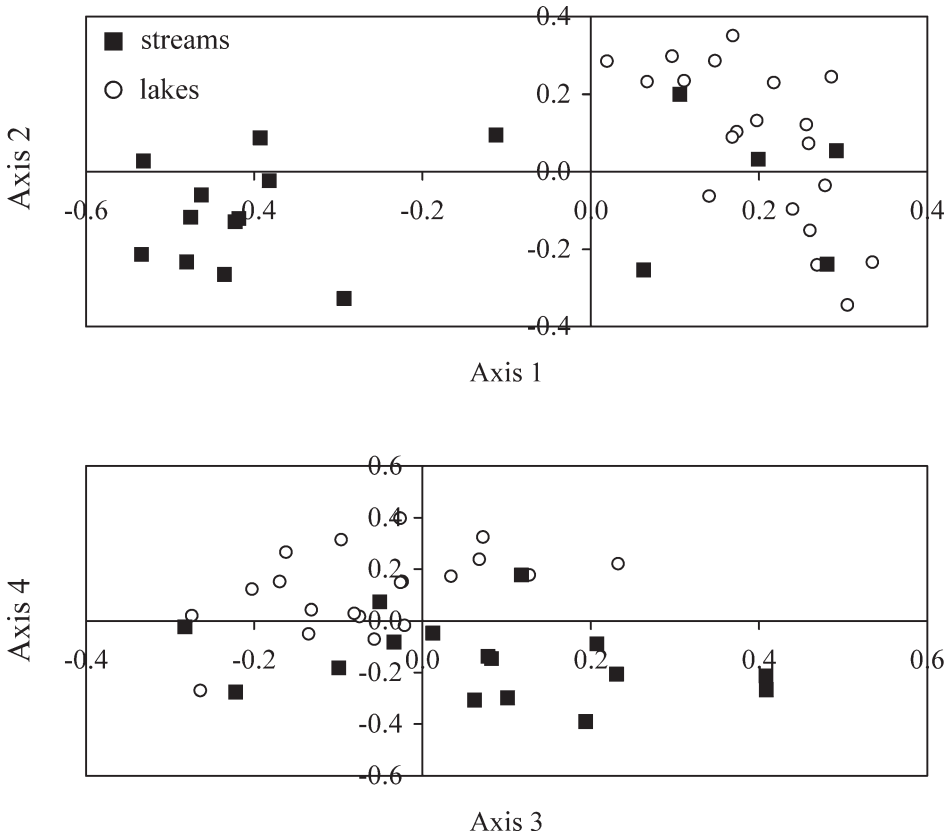

Fig. 1. Results from multidimensional scaling (MDS) of all tRFLP data. The solution with four axes is displayed. Kruskal's stress = 0.103.

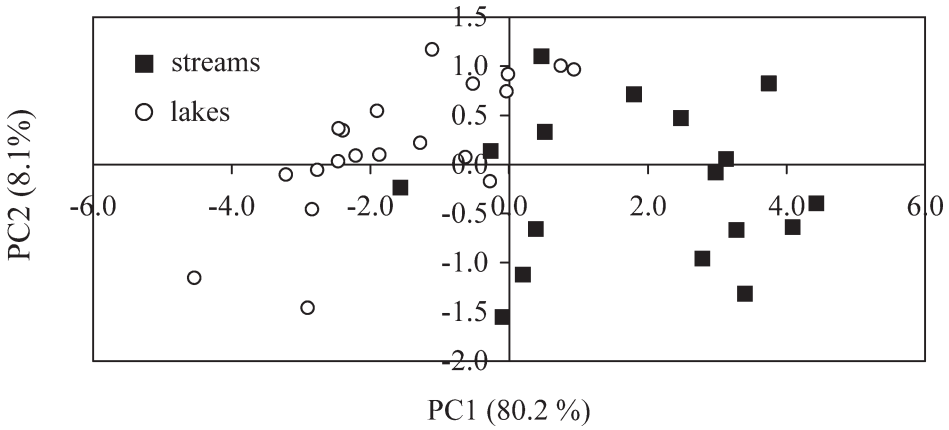

Fig. 2. Results from principal components analysis (PCA) of environmental data from lakes and streams. The first two axes are shown.

phorus compared with the lakes, but the ratios of carbon per P or N unit did not differ. pH was lower in the streams, and water color (expressed as Abs_{280} and Abs_{440}) was higher. The spectral slope also differed between lakes and streams.

Finally, water retention time estimates for lakes and streams differed greatly (Table 1). While already 5 m^2 is a great overestimation of the average cross section area of the streams, which produced an average retention time of 9 d as compared to 350 d for the lakes, the average cross section area had to be set to 110 m^2 to yield a similar average water retention time for streams as for lakes. Hence it is a robust conclusion that the retention time of the lakes is several fold higher than that of the streams, and, assuming that bacterial abundances were in the same order of magnitude, bacterial transport to the streams must be much higher than in the lakes.

BP : DOC in lakes and streams was negatively correlated to PC1 for environmental data as shown by Spearman rank correlation analysis (Table 3). This axis constituted 80.2% of the variation in the environmental data set and mainly represented dissolved nitrogen, phosphorus, and carbon, Abs_{280}, and Abs_{440}, i.e., when all these variables showed high values BP : DOC was low. Further BP : DOC was correlated to those MDS axes of BCC data that differed to the greatest extent between lakes and streams, i.e., MDS1 and MDS4 (Tables 1 and 3). Thus, the difference in function between lakes and streams could be attributed both to differences in BCC between lakes and streams and to differences in the environment.

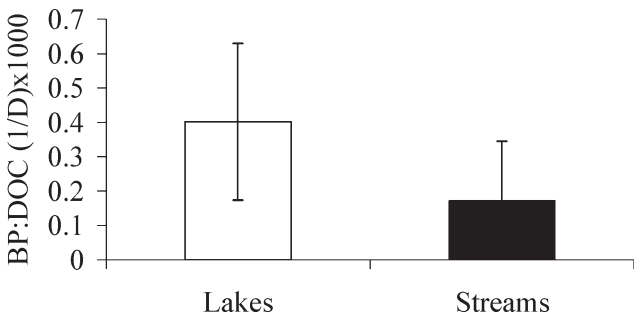

Fig. 3. BP : DOC in lakes and streams. The bars show mean of 20 lakes and 17 streams, and the error bars show the standard deviation.

PC1 was in turn correlated to both MDS1 and MDS4. Partial correlations showed that PC1 was correlated to BP : DOC independent of MDS1 and MDS4 (Table 4), i.e., there was evidence for a connection between function and environment independent of BCC.

Furthermore, MDS1 was significantly correlated to BP : DOC independent of PC1. Thus, there was also evidence for a connection between function and BCC independent of the environment. However, the correlation between MDS4 and BP : DOC was no longer significant when controlling for PC1.

Results from ANCOVA show that a model including PC1, MDS1, and a dummy variable representing lake or stream could significantly explain close to 40% (46% for ranked data) of the variation in BP : DOC among the sampled lakes and streams (Table 5). Further, the p values showed that the lake–stream dichotomy provided little explanatory power to the model once the contribution of PC1 and MDS1 was accounted for. Thus, the fact that BP : DOC was lower in the streams compared to the lakes was mostly a result of BCC and the environment differing in the two types of habitats, and not due to any other difference between lakes and streams, such as e.g., the water flow rate in itself. In this model MDS1 was the most important variable. For ranked data, MDS1 was still significant ($p < 0.05$) when the contribution by PC1 and the lake–stream dichotomy was accounted for. PC1 was in this case close to significant.

Analyzing only stream samples, BP : DOC was significantly correlated to a BCC axis (MDS3) and to the environmental axis PC4. PC4 constituted 6.1% of the variation in the environmental data and represented mainly dissolved phosphorus. Thus, to some extent a high DP contributed to a high BP : DOC. PC4 and MDS3 were not significantly correlated to each other ($p = 0.79$). Thus, there was evidence of BCC as well as environment being of importance for function, independently of each other, in the streams.

Analyzing only lake samples, BP : DOC was not significantly correlated to any MDS or PC axes (Table 3).

Table 3. Results from Spearman rank correlation analyses between BP : DOC and PC axes representing E data and MDS axes representing BCC.

Data set	Significant variables
Lakes	none
Streams	MDS3 ($p=0.036$, $\rho=0.515$), PC4 ($p=0.026$, $\rho=0.544$)
All data	MDS1 ($p=0.0004$, $\rho=0.558$), MDS4 ($p=0.025$, $\rho=0.371$), PC1 ($p=0.0005$, $\rho=-0.552$)

Thus, variation in function among the lakes appeared independent of both BCC and the environmental variables included in the models.

Discussion

The aim of this study was to contribute to the conceptual understanding of what is determining bacterial functionality and the coupling between bacterial function and BCC in inland water ecosystems. We hypothesized that bacterial communities experiencing different water flow regimes would also differ in an important bacterial carbon processing function (BP : DOC), either because of differences in the environment, BCC, or the mechanism shaping BCC (i.e., in the importance of bacterial dispersal).

The water retention time of the streams was much shorter than in the lakes, i.e., water flow regime differed greatly. Thus, a comparison between the lakes and the streams seemed suitable for testing of our hypotheses. BP : DOC was higher in lakes than in the streams (Fig. 3), i.e., we could not reject our hypothesis about a difference in functionality depending on water flow regime. BP : DOC in lakes and streams was, however, also related to the environment (independent of BCC) in the lakes and streams. Thus, the different environment in the streams may have contributed to the lower BP : DOC there, for instance because of the more strongly colored DOM of the streams, which can have been more recalcitrant to degradation. For instance, a high SA_{280} may reflect aromaticity (Chin et al. 1994), and lower spectral slope may be attributed to differences in DOM quality such as higher molecular weight (Helms et al. 2008), both of which can have an effect on degradability. In agreement with these results, Judd et al. (2006) found that stream DOM could suppress bacterial production compared to lake DOM.

The difference in BP : DOC between lakes and streams also seemed to depend on differences in BCC independent of the environment, i.e., different bacteria may have been differently capable of degrading the DOM and to use it for biomass production (Martinez et al. 1996; Cottrell and Kirchman 2000). However, we found no evidence for a lower BP : DOC in the streams *independent* of BCC and environment (Table 5), and, thus, dispersal rate did not seem to have affected functionality per se. The explanation to this result can be that, despite the fact that the lakes and streams differed in water flow regime, and thus most likely in cell dispersal rate, species sorting as opposed to mass effect was equally important in shaping BCC in both lakes and streams. This conclusion is based on the fact that BCC was about equally well explained by E variables in both data sets but never by spatial predictors (Table 2), which supports species sorting but not mass effect (Cottenie 2005). An important question in this context is how high dispersal rates to inland waters are required to cause mass effects (Logue and Lindström 2010) that could potentially have influence on functionality as seen in the laboratory model by Venail et al. (2008), where dispersal rates were up to 100% of biomass per day.

Table 4. Results from partial spearman rank correlations analyses between BP : DOC and significant PC and MDS axes (Table 3). Only the data set including both lakes and streams was analyzed.

Explanatory variable	Controlling variable	p	ρ
MDS1	PC1	0.003	0.471
MDS4	PC1	0.57	0.099
PC1	MDS1	0.004	−0.462
PC1	MDS4	0.006	−0.449
PC1	MDS1, MDS4	0.036	−0.356

Table 5. Results from ANCOVA, testing the importance of the different variables being significant in the partial correlation (Table 4), as well as the lake–stream dichotomy for BP : DOC in lakes and streams. The ANCOVA was run with original data as well as ranked data.

	Original data	Ranked data
Full model	$r^2=0.396$, $p=0.001$	$r^2=0.460$, $p=0.0001$
MDS1	$p=0.055$	$p=0.025$
PC1	$p=0.268$	$p=0.066$
Lake or stream	$p=0.694$	$p=0.766$

To summarize thus far, we found that functionality was lower in streams than in lakes, which seemed to be due to differences in the environment, but also in BCC between lakes and streams. Clearly, such findings support the idea of importance of bacterial diversity for function, which is valuable information, but not a new finding (Langenheder et al. 2006; Bertilsson et al. 2007). However, data on the coupling between BCC and function in the field are generally lacking, and especially, we lack information about under which circumstances BCC should be of importance for function, and under which circumstances BCC should be unimportant.

To shed some light on the latter aspect, we split our data set into two, analyzing lakes and streams separately. We found that there was a great difference between lakes and streams in the relationship between BCC and function, since a covariation between BCC and function was only found among the streams. Lakes and streams seemed similar regarding the relative importance of species sorting and mass effect shaping BCC, as reported above. However, a large part of the variation in BCC among lakes and streams, respectively, could not be explained by species sorting or mass effect (Table 2). That a large portion of variation in BCC remains unexplained is a quite common phenomenon in studies of this kind (Beisner et al. 2006; Van Der Gucht et al. 2007). In a previous study we found that the abundance of certain taxa in our lakes or streams could to a great extent depend on the abundance of that taxon in the other lakes or streams in the region (Östman et al. 2010). We termed this

phenomenon ''regional invariance'' (Östman et al. 2010), and it may be the result of communities to a great extent being shaped by neutral processes (Sloan et al. 2006). Neutrality is a reflection of functional equivalence among trophically similar taxa and is hence strongly conceptually connected to functional redundancy. Thus, it can be expected that the greater the regional invariance the greater the functional redundancy, and the weaker the connection between BCC and function. This is in line with our findings here of the weaker BCC-function coupling among the lakes, which showed a greater regional invariance compared with the streams (Östman et al. 2010). In the previous paper (Östman et al. 2010), we found that smaller environmental variation led to greater regional invariance. Considering the results from the present study it can therefore be expected that the greater environmental variation among sites (i.e., in our case the streams), the stronger the coupling between BCC and function, which was also shown by Comte and del Giorgio (2010).

To summarize, we, like Comte and del Giorgio (2010), conclude that it is not a matter of *if* diversity and function are coupled but rather *when* and *how*. For instance, since diversity–function relationships depend on the functional variable measured (Langenheder et al. 2006; Gamfeldt et al. 2008), the results obtained here cannot be expected to be valid for all important functions bacterioplankton play in inland waters. Our results, however, imply that future studies designed to investigate the possible covariation between BCC and function need to consider that function may depend on a close interplay between BCC and environment. Further, assembly processes may play a role for function, although our data did not suggest an importance of dispersal rate, but rather that the possible role of neutrality and functional redundancy among communities should be evaluated.

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