Biogeography of bacteria in surface sediments of a shallow lake

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Abstract:
Research on biogeographic patterns of bacterial community composition (BCC) has largely concentrated on planktonic bacteria and marine sediment bacteria although bacteria in surface sediments of fresh water lakes may also exhibit biogeographic patterns. Therefore the study of sediment bacterial communities will improve the understanding of BCC patterns in surface sediments of freshwater lakes. It can also help to comprehend the biological processes such as dispersal as well as the influence of environmental parameters on BCC over different seasons. This study evaluated the spatial patterns in BCC change within surface sediments of Lake Lumpen, Sweden, using terminal restriction fragment length polymorphism (t-RFLP). I demonstrated that there was a strong influence of either contemporary environmental condition or spatial scale on the community compositions in different seasons and also the patterns in BCC changed differently over spatial distance and seasons. This result could be an important step to gain insight into seasonal factors shaping BCC in surface sediments and also of shallow lakes.

1. Introduction

1.1 General Background

One major goal of ecology is to understand and measure biogeographic patterns. Biogeography is the study of 'who is where and at what abundance and why? providing knowledge about ecological processes and mechanisms that maintain diversity and shape community composition (Martiny et al., 2006) . Earth contains 70% of water in its surface, of which 3% is fresh water, which is as important as marine water from an ecological point of view. Different types of communities and ecosystems are present under different life zones of freshwater and they are different from ocean water. From this it can be assumed that fresh water bodies may also contain very important biogeographic patterns of microbes. Earth, the so called 'water planet', is teeming with microorganisms, which have a mass of $<10^{-5}$ g and a length <500 μm (Madigan & Martinko, 2006) and are almost invisible to our naked eyes. It has been estimated that there are in total up to 10^9 microbial species on Earth (Pedrós-Alió, 2006) and more than a million microbial cells in one milliliter of water (Whitman et al., 1998). The biogeography also helps to understand the roles of microbes in numerous ecological processes on earth (Grundmann, 2004; Zehr, 2010). Therefore, biogeography can be used in the work of protect, preserve and sustainably use specific genes, species and communities of microbes.

Box 1. Definitions

Community Ecology: Study of interactions among interdependent species cohabiting the same geographical area and insights into how the environment, their interaction and mechanisms affect the abundance, functions, diversity and distribution within and between the communities (Johnson & Stinchcomb, 2007).

Aquatic life zones: The layers of freshwater bodies where various aquatic ecosystems and communities of organisms live. Different life zones of freshwater are littorial, limnetic, profundal and benthic zone.

β-Diversity: A difference in community compositions among ecosystems or sites (Lomolino et al., 2006; Lindström & Langenheder, 2011).

Biogeography: Distribution of biodiversity over space and time (Lomolino et al., 2006; Martiny et al., 2006), aims to insight into the mechanisms such as extinction, speciation, dispersal, colonization and biotic interactions to create and maintain the diversity (Brown & Lomolino,1998; Bell et al.,2005b; Whitfield 2005; Lindström & Langenheder, 2011).

Provinces: An area with specific biotic community due the result of the historic events (Martiny et al., 2006).

Dispersal: Movement of individual from one site to another site (emigration and immigration).

Terminal Restriction Fragment Length Polymorphism (t-RFLP): - A fingerprinting technique based on the restriction digestion of double stranded fluorescently end labeled PCR fragment, useful for estimation of bacterial community compositions.

1.2 Hypotheses in microbial biogeography

Macroorganisms' biogeography has been well studied since Darwin (O'Malley, 2007) but it is less studied in microorganisms, maybe because of little knowledge about factors shaping the community composition. The study of biogeography of microorganisms has begun with the very popular slogan "everything is everywhere: but the environment selects" (Baas Becking, 1934) leading to a focus on the importance of environmental conditions for microbial distribution patterns (O'Malley, 2007). However, to obtain more insight into the causes of distribution patterns of microorganisms, four hypotheses have been presented: null hypothesis (random distribution), influence of multiple habitats conditions (contemporary environmental variations), influence of multiple provinces within one habitat type (influence of spatial variation due the historical events) and influence of multiple habitats and multiple provinces (Martiny et al., 2006). According to these hypotheses, an increase in environmental similarity and/or a decrease in geographical distance between the sites create the possibility of similar community compositions. Thus, not only the importance of local contemporary environmental conditions should be considered in biogeography research.

1.3 Metacommunity theory in biogeography

Another theoretical concept, the metacommunity concept, deals with a set of local communities interconnected by dispersal at different spatial scales proposing four scenarios; species sorting, patch-dynamics, mass effect, and neutral perspective (Leibold et al., 2004). This concept was developed for larger organisms, but might be of importance for microorganisms as well (Logue & Lindström, 2008; Lindström & Langenheder, 2011). Species sorting emphasizes the patch heterogeneity, i.e. differences in the local habitat conditions cause differences in the local communities assuming dispersal to be unhindered. Patch dynamics assumes that identical patches, capable of containing populations, may or may not be occupied and the empty patches might be colonized by dispersal from a new patch. Therefore spatial dynamics might be determined by local extinction, dispersal and colonization. Mass effect emphasizes the effect of immigration and emigration on local population dynamics where suboptimal competitors can be rescued from extinction due to immigration. The neutral paradigm anticipates that all species are similar in their competitive ability, mobility and fitness; therefore the community dynamics are derived from probabilities of species loss (extinction, emigration) and gain (immigration, speciation). This perspective therefore assumes that only geographical distance is related to community composition.

1.4 Bacterial communities in surface sediments

Bacterial community composition (BCC) in the surface sediments of small freshwater lakes is not so much studied compared to that of the water column or compared to marine sediments. Several studies of bacterial communities in sediments of marine habitats have been conducted (Inagaki et al., 2002, Hewson & Fuhrman, 2006; Franco et al., 2007). For example, BCC in the sediment surface of the South Atlantic Ocean at intermediate spatial scales (10-3000km) and large spatial scales (>3000km) has been shown to be a result of interplay between local contemporary environmental factors but it was a result of dispersal limitation and spatial distances in small scales (< 200m) (Schauer et al., 2010). However,, some studies in sediment of freshwater lakes have studied the effect of organic aggregation (Tang et al., 2010) and seasonal variability (Tserttova et al, 2011) on BCC and vertical distribution of BCC in sediment (Ye et al., 2009). For example, two genetically distinct sulfur bacteria (*Achromatium sp.)* communities recorded from the lake sediments of North England and Germany reflect the result of species sorting (Gray et al., 2007). So it could be important to know the habitat preferences and factors for dispersal in particular surface sediments. Heavy

precipitation (rainfall or snow fall), fetch (exposed water surface for traveling air), surface ice cover, seasonal water circulation and anthropogenic activities (fishing, boating, and overexploitation) in lakes may also promote dispersal, affecting the community compositions (Wetzel, 1990a). Especially in shallow lakes, the extent of resuspension into the water column transports sediment with microorganisms to different locations resulting into uneven distribution of microbial population (Steinberg, 1978b). But it can also lead to homogenization in community composition especially in small and shallow lakes. Therefore it could be assumed that BCC might not be strongly affected by spatial distance in shallow lakes but might be greatly structured by local environmental conditions (e.g. Logue, 2010). Due to that reason, Lindström & Langenheder (2011) recommended that potential dispersal factors for specific habitats should be well determined for studies of microbial biogeography.

1.5 About the project

Since sediment bacteria are probably less mobile than planktonic bacteria and therefore communities mix to a low extent, it is quite possible that the former communities vary to a greater extent than the latter. Further, it is also possible that the community composition of sediment bacteria is to a greater extent determined by historical and dispersal factors than by local environmental forces, since the difficulties to move around may introduce a stochastic factor in community assembly. Still it is unclear on which scales communities vary, for instance if communities only millimeters apart are more similar than communities decimeters apart, meters apart and so on. In addition, there is no information available if biogeographic patterns change with time, depending on for instance resuspension rates, which in turn depend on wind mixing, which depends for instance if the lake is ice covered or not. Therefore in this project, biogeography of sediment bacteria was studied on different spatial scales within Lake Lumpen, Uppsala County, Sweden. The study was carried out at three times, late spring in June, summer in August and winter in January to evaluate if there is a temporal change in biogeographic patterns. The factors that influence the community composition were distinguished by multivariate statistics. For determination of community composition of the sediment bacteria terminal restriction fragment length polymorphism (t-RFLP) was used. The main aim of this study is to obtain insight into the factors influencing the BCC in surface sediments of the shallow lake, if the community compositions change over time. For this study the formulated hypotheses were;

(1). The closer the geographic distance, the more similar the bacterial community compositions. BCC may be shaped by small differences in sediment environmental characteristics or by local dispersal.

(2). Biogeographic patterns change with seasons. At periods of little mixing local dispersal may better explain patterns in BCC than environmental conditions due to dispersal limitation. At periods of greater mixing homogenization may occur.

2. Methods and materials

2.1 Study lake and sampling stations

The study lake, lake Lumpen, is a roughly triangular shaped lake located in the geographical coordinates of 59°58'N and 17°17'E, in Uppsala County, Sweden. The lake is small, about 0.25 km^2 in area and shallow, maximum depth 1.9 meters. The lake volume is about 0.31×10^6 m^3 and water retention time (WRT) is about 164 days. The lake is fed by an inlet in the north-west corner and has an outlet in the south-east corner. The sediment samplings were carried out three times, 21st of June 2011 (late spring), 24th of August 2011 (summer), and 18th of January 2012 (winter) at eleven stations. Station A was close to the outlet, station H was close to inlet, J and K were in the third corner of the lake and the remaining was in middle of the lake as shown in Figure 1. The sampling stations were chosen with different geographic distances among them in three different directions according to the shape of lake. All sampling stations A to K were marked by the GPS (GARMIN, Germany) for geographical coordinates, and the data was transferred for mapping the stations in computer software, Arc GIS (Fig.1).
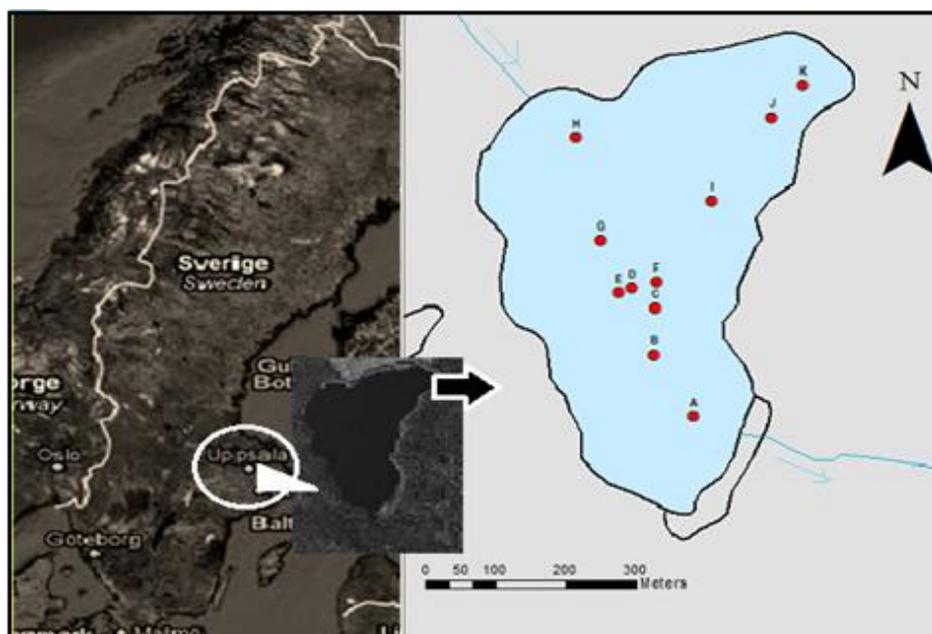
Fig. 1 Sampling stations A to K according the geographic coordinates in Lake Lumpen, (21st June, 2011). Background maps from google and the "Lantmäteriet" (© Lantmäteriet Gävle (2010): Permisson I 2010/0058).

2.2 Physicochemical parameters of the lake

From all stations temperature and oxygen concentration were measured at the sediment surface using the Oxygen and Temperature Probe (Oxi 340/SET, WTC Wissenschaftlich Technische Werkstätten GmbH, Germany) and depth was also measured before sampling the sediment (Table.1). Temperature and oxygen depth profiles were measured from the central part of the lake (Table 2).

2.3 Sediment samplings and treatment

A sediment core for each station was collected using a sediment sampler (tube of 40cm long and 4 cm diameter). For each station, three subsamples were collected semi-randomly (i.e. from three different positions of the core) for BCC by drawing 1 ml of sediment from the upper first cm of the core. The remaining upper first cm was sliced into a jar for bacterial abundance and sediment chemistry analysis. All samples were immediately stored in a cooler box, followed by transport to the laboratory. In the lab the vials for BCC were stored at -80

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°C until the extraction of DNA and the samples for sediment chemistry analysis was stored at -20 °C. Prior to freezing 0.5 ml of samples was taken out from each of the sample jars for bacterial abundance analysis. In June, sample E was lost.

2.4 Bacterial abundance

0.5 ml of each sample was diluted 100x with a 50/50 mixture of tap water and deionized water, preserved with 5 ml formaldehyde (2-4% final concentration) and stored at ±4 °C. The samples were again diluted 50x from which 10ml was taken for sonication. After sonication, 2ml sample with 3ml of Acridine Orange solution (final concentration 0.01 %) were loaded to the filter equipment for filtration. The prepared slides were finally analysed using fluorescence microscopy (blue excitation, Eclipse €600, Nikon, Japan) (Hobbie et al. 1977).

2.5 Sediment chemistry analysis

For the analysis of sediment chemistry, Total Carbon (TC), Total Nitrogen (TN), and Total Phosphorus (TP) by percentage (%) dry weight, the samples were defrosted and freeze dried (Edwards Manifest 680, Edwards, West Sussex, England) and homogenized. Then a Costech ECS 4010 elemental analyser (Costech International S.P.A., Italy) was used for computing the % dry weight of total carbon and total nitrogen in sediment samples. Total phosphorus (% dry weight) was analyzed at absorbance of 882 nm using spectrophotometry (Hitachi U-2000, Hitachi Ltd, Tokyo, Japan) (Andersen, 1976; Menzel & Corwin, 1965).

2.6 Bacterial community composition by t-RFLP

Analysis of bacterial community composition (BCC) was carried out by the fingerprinting methodology terminal Restriction Fragment Length Polymorphism (t-RFLP), using the restriction enzyme *Hae III* on the basis of 16S ribosomal ribonucleic acid (rRNA) gene.

2.6.1 DNA extraction

Total community DNA was extracted from 0.5 ml of wet sediment using the Ultra Clean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, USA).The final elution volume of DNA was 100 µl. The extracted genomic DNA quality was verified by running the products on a 1% agarose gel. After verification, the extracted DNA solutions were stored at -20 °C for further processing.

2.6.2 Polymerase Chain Reaction (PCR)

2 µl of extracted DNA was added to 18 µl PCR-mixture containing 5-carboxyfluorescein labeled forward primer Eub 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer Eub 519r (5'-GWATTACCGCGGCKGCTG-3') (Turner et al., 1999) according to Bioline Ltd. Meridian Life Science Company, UK. The samples then run for PCR to 30 cycles through steps of denaturation at 94 °C for 30 sec., annealing at 52 °C for 30 sec., extension at 72 °C for 45 sec., final extension at 72 °C for 7 min. and cooling at 4 °C for infinite time. The PCR products were stored at -20 °C. To collect enough PCR products for further processing, PCR was repeated with double the volume of extraction and PCR-mixture. Each PCR run was verified by running the products on a 1% agarose gel.

2.6.3 Cleaning up and quantification of PCR products

The PCR products were cleaned using AcroPrep TM 96-well filter plates 30K Omega (Pall Life Science, Pall Corporation, USA) and stored at -20 °C. The PCR cleaning was followed by DNA quantification. The DNA quantification for each sample was done according to the protocol of Quant-iT TM PicoGreen® (Invitrogen, Life Technologies Ltd, Oregon, USA) Assay,catalog number: p7589, creating the standard curve of lambda (λ)-DNA.

2.6.4 Restriction enzyme digestion (R-digestion)

Prior to R-digestion, 5 µl of the cleaned and quantified PCR products with a concentration of 10ng/µl were mixed with 5 µl of mastermix (containing 1 µl buffer4, 3.6 µl MQ water and 0.4 µl *HaeIII* restriction enzyme) to get a final volume of 10 µl per reaction. Each sample in duplicates and two negative controls were incubated at 37 °C for 16 hours at moist condition for restriction digestion. The separation of restricted fragments was carried out in Uppsala Genome Centre of Rudbeck Laboratory, Uppsala, Sweden using ABI3730XL DNA Analyzer on the basis of fluorescently HEX-labeled fragments running in GeneScan mode. The peak fragments less than 50 base pairs (bp) and greater than 500 bp were eliminated. Peaks less than 0.5 bases apart were merged. Peaks greater than detection threshold of 90 and present in duplicates of a sample were only considered as peaks. Further, a table containing all peaks and the intensities of the terminal fragments was generated by Genotype software, Genemarker (Version 1.70).

2.6.5 Treatment of electropherogram data

The table of the terminal fragments from Genemarker was transported to excel to create data sets of absolute peak intensity, relative peak intensity and number of peaks (i.e. presence/absence) at thresholds of 0 % (means original), 0.5 % and 1 %. The 0.5% threshold was used for June and January samplings and data set at 1% threshold was used for August sampling for further statistical analysis.

2.7 Data handling and Statistical analysis

The data sets of sediment chemistry and bacterial abundance for all sampling stations were analyzed by correlation analysis using the R software to show the change in bacterial abundance in relation to sediment nutrient of the station. For this analysis, log transformed data of sediment chemistry was used. Principle component analysis (PCA) was carried out to illustrate the covariation between sediment chemistry variables (TC, TN and TP) using PAST (Paleontological Statistics) software (version 2.03). For this, sediment chemistry data was transformed to Z- scores values after log transformation.

To evaluate the bacterial community composition, dissimilarity (Bray-Curtis distance) was calculated and analyzed by nMDS using PAST (version 2.03). Mantel tests were performed to check the influence of spatial distance and sediment chemistry variables on the bacterial community composition. Partial Mantel test were run to analyze the relationship between community composition and environment correcting for geographic distance and then the relationship between community composition and geographic distance correcting for environment. For these tests, the geographical distance matrix of sampling stations and Euclidean distance for environmental variables (TC, TN, and TP) were calculated in Arc GIS computer software and PAST (Paleontological Statistics) software (version 2.03) respectively. For partial mantel test, TC was considered as sediment environmental factor as it showed significant correlation to BCC in mantel test. Both mantel test and partial mantel test were both carried out using the R software (2.13.1 version).

To analyze if the biogeographic patterns in BCC change over time (the three seasons), the mantel tests were run comparing the Bray-Curtis dissimilarity matrices from the different seasons. Also, to analyze whether bacterial communities within sediment cores were more similar to each other than to communities from other cores permutational ANOVAs were run using the ADONIS function in R.

3. Results
3.1 Physicochemical characteristics of Lake Lumpen

Geographic location, temperature and oxygen concentration of all stations during the study period is shown in Table 1. Depth of sampling was only measured in August and January and increased slightly in January. The sampling depth ranged from 1 m to 1.8 m. Temperature showed a slight increase of almost 1°C in all stations from 16.3 °C in June to 17.7 °C in August except in stations J and K where it seemed same. Temperature was low in January (i.e. winter sampling), ranging between 3°C and 4.2 °C. Oxygen concentration was 3 mg/l to 6 mg/l in June except in station G (0.2 mg/l). In August, oxygen concentration varied from 3 mg/l to 7 mg/l, being higher than in June (except for station F). In January, oxygen concentration was below 1 mg/l in all stations (varied between 0.4 and 0.8 mg/l).

Table 1 Geographical coordinates depth, temperature and oxygen concentration of all stations

|	Geographic coordinates		June 2011		August 2011			January 2012		
	WGS 84	WGS 84	Temp.	Oxygen	Depth	Temp.	Oxygen	Depth	Temp.	Oxygen
Stations	Lat. (N)	Long. (E)	(°c)	(mg/l)	(m.)	(°c)	(mg/l)	(m.)	(°c)	(mg/l)
A	59 57.441	17 16.752	16.5	3	1.5	17.5	6	1.6	3.2	0.4
B	59 57.498	17 16.690	16.6	6	1.5	17.5	6	1.7	4	0.4
C	59 57.542	17 16.736	16.3	6	1.5	17.4	5	1.8	3.9	0.5
D	59 57.540	17 16.693	16.3	6	1.5	17.3	6.4	1.8	4.3	0.8
E	59 57.550	17 16.685	16.4	5.8	1.5	17.3	5	1.8	4.2	0.6
F	59 57.549	17 16.772	16.4	5.8	1.5	17.4	0.2	1.8	4	0.5
G	59 57.559	17 16.649	16.4	6	1.5	17.3	3	1.8	4	0.6
H	59 57.659	17 16.590	16.4	0.2	1	17.7	7	1.5	3.6	0.7
I	59 57.671	17 16.860	16.5	3	1.5	17.4	5	1.8	4.5	0.8
J	59 57.680	17 16.922	16.9	4	1	17.3	6	1.5	3.8	0.6
K	59 57.714	17 16.975	17.2	5	1	17.1	7	1.5	3	0.8

This temperature and oxygen profiles was recorded at the time of sampling for August and January but for June it was taken from record of my research training (9th June 2011). Measurement of temperature and oxygen concentration from four depths illustrated that surface temperature was higher in June and August but the reverse was the case in January (Table 2). The surface and bottom temperature recorded during the study period were 21.5 °C and 19.6 °C in June, 17.3 °C and 18.3 °C in August and 0.4 °C and 4 °C in January. The record indicates that the lake was thermally stratified at all sampling occasions and that surface water was mixed down to 1 m depth in summer and down to 0.5 m in winter. i.e. oxygen concentration varied from 8.23 mg/l at the surface to 0.12 mg/l at the bottom in June and August. In January, oxygen concentrations were higher (18 mg/l in at the surface and 2.7 mg/l at the bottom).

Table 2 Temperature – Oxygen profiles in the three different seasons

	June 2011		August 2011		January 2012	
Water Depth	Temp.(°C)	O_2 (mg/l)	Temp.(°C)	O_2 (mg/l)	Temp.(°C)	O_2 (mg/l)
0 m.	21.5	7.13	18.3	8.23	0.4	18
0.5 m.	21.3	6.8	17.7	8	1.5	16
1 m.	21.2	5.98	17.3	8.05	3.1	5.8
1.5 m.	19.6	0.12	17.3	0.13	4	2.7

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3.2 Sediment environment of the stations

Principal component analysis was used to figure out the relationships between the environmental variables, total carbon, total nitrogen and total phosphorus (fig.2). The principal component analysis (PCA) showed that first axis of sediment chemistry variables in June and August 2011 explained 55.5 % to 64.1% of the total variation, whereas second axis explained 31.5 % and 34.9% and third explained 4.4 % and 9.7 % in June and August, respectively. In January 2012, first axis explained 47.9%, second axis explained 31.1% and third axis explained 21% of total environmental variables of sediments. The PCA demonstrated that TC and TN and covaried in late spring and summer but were not correlated to TP. In winter TC and TP covaried but they were not correlated to TN.
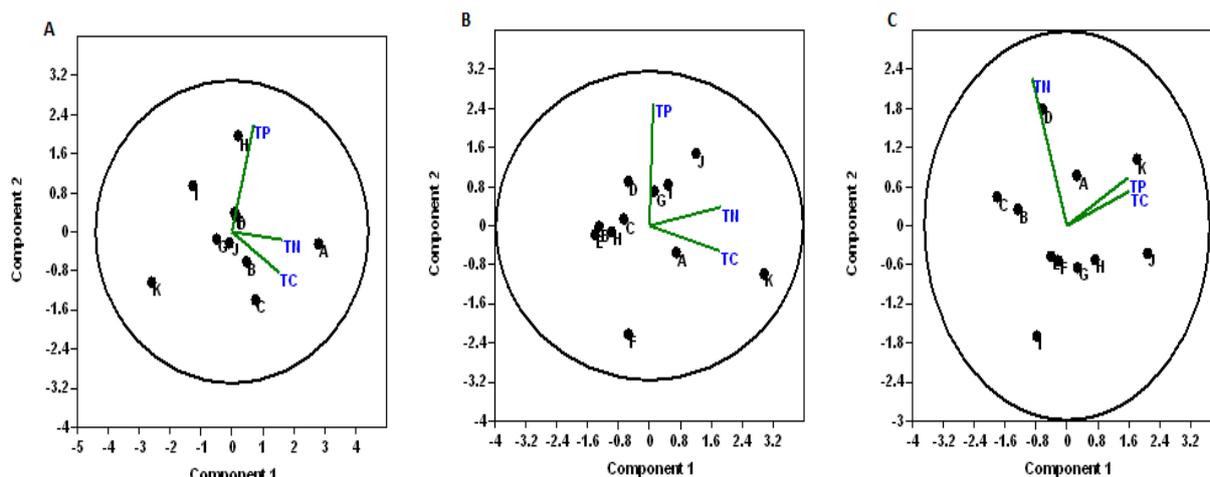
Fig. 2 Results from principle component analysis of TC, TN and TP. The first two axes are shown for June (A) August (B) and January (C) Green lines are environmental factors of the sediment and sampled stations are shown as black dots labeled A-K.

3.3 Bacterial abundance correlation to sediment environment

Bacterial abundance showed a significant positive correlation with TC and TN in June but it was not significantly correlated with TP. In August and January there was no correlation between bacterial abundance and any of the measured nutrient variables (Table 3).

Table 3 Correlation between bacterial abundance and sediment chemistry of total carbon, total nitrogen and total phosphorus.

Sediment	June 2011		August 2011		January 2012	
Chemistry	Untransformed	Log transformed	Untransformed	Log transformed	Untransformed	Log transformed
TC (% dry wt.)	r=0.90780 (0.00028)*	r=0.90556 (0.00031)*	r=-0.03369 (0.9217)	r=-0.01982 (0.9539)	r=0.20022 (0.555)	r=0.20608 (0.5432)
TN (% dry wt.)	r=0.67060 (0.03381)*	r=0.65396 (0.04025)*	r=0.38091 (0.2478)	r=0.38197 (0.2464)	r=-0.08854 (0.7957)	r=-0.08056 (0.8138)
TP (% dry wt.)	r=0.05788 (0.8738)	r=0.06952 (0.8486)	r=0.02153 (0.9499)	r=0.00791 (0.9816)	r=0.28802 (0.3904)	r=0.29607 (0.3767)

p-value inside brackets, asterisk (*) means significant correlation

3.4 Variation in BCC over space at the different sampling occasions

Non-metric Multidimensional Scaling (nMDS) based on Bray-Curtis dissimilarities shows variation in bacterial community compositions among the sampling stations. Closeness to each other means more similar communities and far from each other less similar in community composition. In June A, H and K showed differences compared to the other communities (Fig. 3A). These three stations were far away from each other geographically as well. In August the trends were less evident but station A and K were fairly different from

other stations (fig. 3B). In January, station A, H and K also showed some compositional difference, while along with fair a greater distance among stations I and J and the other sampling stations was observed (fig. 3C). In all seasons, station A and K were clearly different from the other stations.
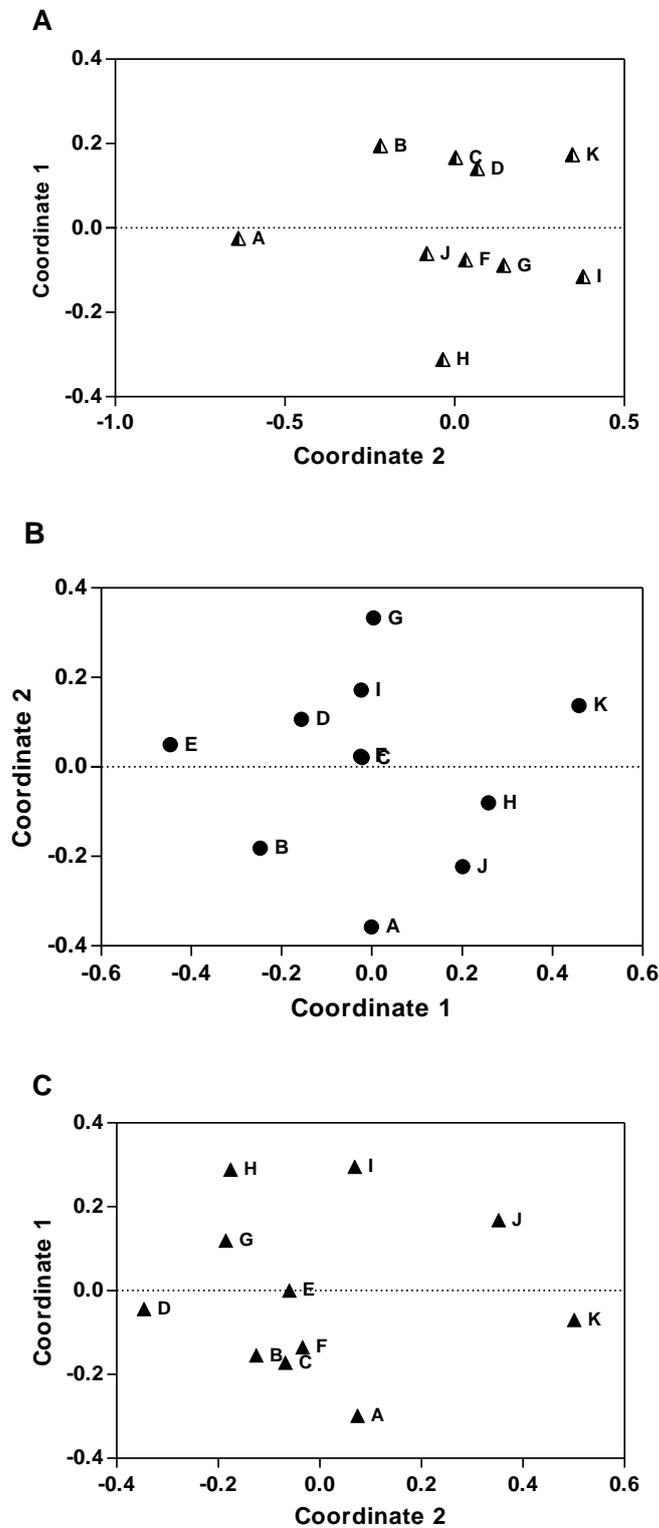
Fig. 3 Non-metric Multidimensional Scaling of bacterial community composition based on means of triplicates of each station in June (**A**)**,** August (**B**) and January (**C**).

One way permutational ANOVA showed that there was a significantly greater similarity in BCC in replicates within a core than among cores. 66% of variation between the samples in June could be explained by the core ($r^2 = 0.66$), 43% of variation ($r^2 = 0.43$) in August and 61% of variation ($r^2=0.61$,) in January. This shows a clear spatial influence on distribution of the bacterial community compositions in all seasons with the greatest importance in June, then in January and the smallest in August.

3.5 Influence of geographic distance and sediment chemistry on BCC

Mantel test showed a significant correlation between bacterial community composition and geographic distances in the all sampling occasions. Mantel test also showed that BCC was significantly correlated to TN and TC in June and August but not with TP. In January significant correlations of BCC was observed only with TC (Table 4).

Table 4 Mantel test showing the correlation between Bacterial community composition distance matrices from t-RFLP and Geographic distance matrices, Euclidean distance of environmental variable matrices (TP, TN and TC). Partial mantel test showing correlation between bacterial community composition distance matrices with geographical distance controlling for environment and environmental distances controlling for geographic distance.

	Mantel tests				Partial Mantel tests	
Sampling	Geographic distance	Total Phosphorus	Total Nitrogen	Total Carbon	Geographic distance/TC	TC/geographic distance
Jun. 2011	**0.433 (0.024)***	0.166 (0.23)	**0.616 (0.013)***	**0.802 (0.001)***	0.2393 (0.165)	**0.7655 (0.002)***
Aug.2011	**0.392 (0.007)***	-0.2843 (0.91)	**0.2886 (0.032)***	**0.4759 (0.014)***	0.199 (0.152)	0.3497 (0.07)
Jan. 2011	**0.7286 (0.001)***	0.07248 (0.33)	0.223 (0.098)	**0.5968 (0.002)***	**0.57 (0.001)***	0.2713(0.113)

r-values outside brackets, p-values inside brackets. Bold with asterisks (*) indicates significant correlation

Partial mantel tests showed that BCC was strongly correlated to TC independent of geographic distance in June. However, the correlation to geographic distance was not significant when controlling for TC. The opposite result was obtained for the January samples, i.e. BCC was correlated geographic distance independent of TC. For August, there was no positive correlation of BCC to either geographic distance or TC, when correcting for each other in partial Mantel tests (Table 4).

4. Discussion

The aim of this study was to obtain insight into the factors shaping BCC in sediments in shallow lakes. The hypotheses formulated for the study were; 1) BCC may be shaped by small differences in sediment environmental characteristics or by local dispersal. 2) The relationship between BCC and environment or dispersal depends on the season. For this, change of BCC over spatial scales in three different seasons was analyzed performing mantel and partial mantel test of a t-RFLP genetic distance matrix, a geographic distance matrix and an environment distance matrix.

In June, BCC was significantly correlated to total carbon in sediments independent of geographic distance. The correlation to carbon may be probably due to the distribution of different organic compounds in different spatial distances by the result of surface mixing down to bottom and resuspension during the spring season in shallow lakes (Bloesch, 1995 and Hewson et al., 2007) but an uneven distribution of total carbon content in the sediments was not seen. Previously environmental parameters like organic carbon, sediment type and porosity has been shown to shape the microbial communities in sediments (Queric & Soltwedel, 2007; and Jackson & Weeks, 2008).

In August, BCC showed no positive correlation to any of the geographic distance and sediment environment among the stations could be due to less thermal stratification (see Table 2), early autumn strong surface mixing with great resuspension and dispersal among the stations. This may result into the similar sediment environment and homogenization in BCC among the stations. Therefore little variation in BCC could be observed in this season but the variation in BCC will be increased by the winter stagnation and the surface ice cover.

For January, the ice cover period, there was a significant correlation of BCC to geographic distance (Whitaker et al., 2003 and Martiny et al., 2006) even when correcting for the environmental variables i.e. opposite to the result in June. It could be because of winter stratification of water masses causing dispersal limitation and more stable and similar environmental condition during the ice cover period. Similar result has been recorded from the earlier study over small scales (Schauer et al., 2010). The stable environment condition in the lake with no light penetration, wind circulation and human exploitation for longer ice covered period may cause little or no resuspention. This leads to increase of dispersal limitation with influence of geographic distance in BCC.

The closer the geographic locations, the more similar the bacterial community compositions; the hypothesis was supported by the result from permutational ANOVA in all the sampling occasions since within replicates of a core similarity was greater than among the cores similarity. The increase variation in BCC in June and January than in August reveals a greater importance of geographic distance in BCC in early summer and winter than in late summer. Thus, the spatial heterogeneity was highest in June and in January. The low heterogeneity in August could be due to creation of similar environment along with strong surface mixing with resuspension during the early autumn. This finding shows that BCC change differently over spatial distances in different seasons.

The positive correlation of bacterial abundance to total carbon and total nitrogen in June indicates the greater bacterial production with increase of these nutrients in this ice free season. This result is also supported by earlier study of surface sediments across San Petro Basin (Hewson et al., 2007) and in the sediment of Lake Võrtsjärv (Tšertova et al., 2011). No positive correlation of the abundance to any of the nutrients in late summer and winter might be the low penetration of light, low oxygen concentration due to the stratification and surface

ice cover. Although the temperature and oxygen concentration of sediment can alter the bacterial abundance (Sieden et al., 2010), they were not measured in this study.

5. Conclusions

Local contemporary environment, spatial distance with local dispersal are major factors shaping the patterns of BCC in surface sediments of shallow lakes; however specific factor could be determined only in two particular seasons. The patterns in BCC also change over spatial distance in different seasons. This result could be an important step to gain insight into major factors shaping BCC in surface sediments and also help to formulate theoretical concepts about biogeography of sediment bacteria in small and shallow lakes.

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7. References

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