Low seasonal variability in community composition of sediment bacteria in large and shallow lake

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Summary
The community composition of bacteria with highly dynamic abundance and activity was observed to be with low variability in a shallow lake sediment with frequent physical disturbance. This suggests that physical disturbance did not create more niches and did not lead to highly variable bacterial community. The major part of the bacterial community was homogeneous, with 40% of phylotypes being ubiquitous and present in all samples. A minor part was responding to two contrasting periods – permanent ice cover and open lake. During the period of ice cover the total number of phylotypes decreased by ~10%, the productivity of sediment bacteria varied by 15-fold (decreased by 40% under ice) and the abundance of bacteria by up to sixfold (decreased by 20%), suggesting that community of sediment bacteria with stable species composition might be highly dynamic in numbers and activity. Phylotypes identified by 16S rRNA gene sequencing were close to those observed in similar environments. All sequences were closely related only to uncultured phylotypes. Proteobacteria, particularly of the Beta subgroup, were the most common identified species in Lake Võrtsjärv sediment samples. A few other phylotypes, mostly those typical of anoxic sediments, were observed, but were uncommon.

Introduction
Heterotrophic bacteria are among most important contributors to the transformation of organic compounds in many sediment environments, including freshwater lake sediments (Nealson, 1997). In shallow lakes within flat landscape the particulate matter carried along by inflow-rivers precipitates rapidly as the flow velocity falls. In high productive L. Võrtsjärv, the organic particulate matter increases due to productive of phyto-, zoo- and bacterio-plankton and on annual average amount of particulate matter increased by 17% in the lake (Järvet, 2004). In large and shallow lakes of low average depth (<3 m) and long wind fetch (>15 km), direct wave action may induce sediment resuspension as a whole lake event (Bloesch, 1995 and references therein).

In addition, the importance of resuspension may vary depending on the geographical location and climate. Unlike large subtropical and shallow lakes [e.g. Lake Taihu (Nõges et al., 2007; Tang et al., 2009; Ye et al., 2009)], the large shallow lakes in colder temperate zone have permanent ice cover for several months over the winter during which no wind driven and strong resuspension can occur. Thus boreal lakes serve as perfect sites for studying contrasting physical disturbances. The ice-free and, in most cases, relatively warm period from spring to autumn, is comparable with a large variety of lakes and coastal zones with eventual and irregular physical disturbances. Ice-covered periods lasting 3–4 months are comparable with stratified lakes with low physical shear stress at the sediment surface. Several studies have shown that the ice cover affects the release of nutrients in boreal shallow lakes (Niemistö and Horppila, 2007), and the duration length of ice cover might be the key factor for overall biogeochemistry of the system modulated by climate change (Nõges et al., 2007; Nõges and Nõges, 2010). The influence of ice cover on microbial community composition in shallow lakes has rarely been studied. Therefore, important questions remain about how environmental disturbances and patterns in sediment microbial diversity or activity are linked, and how do they affect ecosystem functioning. Several studies have been conducted in shallow marine environments to determine the link between benthic bacterial community structure and composition with environmental parameters (e.g. Franco et al., 2007; Hewson et al., 2007; Böer et al., 2009). In same cases, frequent resuspension events can increase the niche availability and therefore also result in higher complexity and richness of phylotypes in shallow lake sediments (Tang et al., 2010).

The aim of our study was to survey the diversity of bacteria in the upper layer of the sediment in the large and shallow boreal lake Võrtsjärv, and trace the influence of

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disturbances in the surrounding environment. We assumed that the diversity of sediment bacteria had pronounced seasonal dynamics, but was disturbed by frequent resuspension events during ice-free periods. We also expected to observe a pronounced relationship between redox conditions [dissolved oxygen (DO) concentration], bacterial activity and community composition in the sediment, as well as in plankton biomass and productivity in the water column.

Results and discussion

Seasonal trends in sediment parameters

Dissolved oxygen concentration at the sediment surface ranged from 3.0 to 14.1 mg O₂ l⁻¹ (Fig. 1A), while penetration of oxygen into the sediment averaged 3 mm (median), with the deepest penetration depth being 10 mm (December, 2006). Dry weight of the sediment (DW) ranged from 0.021 to 0.081 g DW (g WW)⁻¹ (wet weight of sediment). Statistically higher DW content was observed in the lower (0.5–1 cm) layer compared with the 0–0.5 cm layer (t-test, t = 6.20, P < 0.01). Over most of the summer months, only 1 mm of light-dense and water-rich sediment [< 0.035 g DW (g WW)⁻¹] contained DO, while oxygen was observed below 5 mm in sediment at the beginning of the ice-covered period from December to February (Fig. 1A). DO was only close to depletion by the end of ice-covered period in March. In the water column, the low DO levels (< 5 mg O₂ l⁻¹) have been observed almost every year beneath the ice (Tuvikene et al., 2002). The temperature ranged from 0.8°C to 22.8°C at the sediment surface. Oxygen concentration in sediment was mostly dependent on the water column temperature (r = −0.55, P < 0.05) and oxygen concentration (r = 0.67, P = 0.011). The high penetration range of DO in sediment at the beginning of winter was probably caused by low activities of bacteria [ranging from 0.82 to 2.3 μg C(mg DW h)⁻¹ from December to February BP]. From April to September, significantly higher production values were observed, with a median BP of 5.6 μg C (mg DW h)⁻¹ (Fig. 1B).

We expected occasionally deeper (below several centimetres) oxygen penetration into the sediment after strong resuspension events because of resedimentation
of oxygen enriched but relatively heavy particles over the ice-free period. Our data supported the notion that DO was introduced to deeper sediment during the ice-free period by extensive resuspension and concurrent sedimentation, but not deeper than 1 cm (Fig. 1A). DO concentration profile and penetration depth in sediment were significantly determined by a combination of water column DO concentration and temperature (significant correlations \( P < 0.05 \)). Therefore, no DO was observed below 5 mm over summer and early autumn when DO concentration in water column was relatively low and the temperature high (Fig. 1A).

**Abundance and productivity of bacteria**

The total number of bacteria (TNB) ranged from 3.97 to \( 25.89 \times 10^9 \) cells (g DW\(^{-1}\)) (Fig. 1B), average \( 14.01 \pm 7.21 \) (± SD). Bacterial production (BP) was similar in the upper sediment layer \( [4.03 \pm 3.90 \mu \text{g C (mg DW)}^{-1}] \) compared with the deeper layer \( [3.46 \pm 3.44 \mu \text{g C (mg DW)}^{-1}] \) (± SD). Seasonal dynamics of BP in these two layers co-varied strongly, with correlation coefficient \( r = 0.95, P < 0.01 \). During the period from March to October 2007, the correlation between TNB and BP was strong and significant \( (r = 0.69, P = 0.027) \). The only marginally significant correlation with a parameter measured in the water column was between TNB and Chl a \( (r = 0.55, P = 0.05) \).

Considering the generally high abundance of bacteria in various aquatic sediments, the cell-specific activity of sediment bacteria was relatively low in L. Võrtsjärv, or possibly, only a small fraction of sediment bacterial community was metabolically active (Novitsky, 1987). However, comparing the activity of sediment bacteria in Lake Võrtsjärv with similar habitats, they were not extremely abundant but were relatively active. Probably the most active among similar habitats, e.g. bacteria colonizing wetland leaf litter, are ~100 times more active, with leucine incorporation rates of 100–1000 nmol (g DW h\(^{-1}\)) \cite{Gillies et al. 2006} compared with L. Võrtsjärv 0.21–5.4 nmol (g DW h\(^{-1}\)). Compared with mesotrophic and a relatively deep L. Erken \cite{Haglund et al. 2003}, bacterial activity was in the same order of magnitude \( [0.27–3 \text{ nmol (g DW h)}^{-1}] \); at the same time, the abundance of bacteria in L. Võrtsjärv was several orders of magnitude lower [0.007 to 0.026 \times 10^{10} \text{ cells (g DW)}^{-1}] vs. 2 to \( 11 \times 10^{10} \) cells (g DW\(^{-1}\)), indicating higher cell-specific activity. In deep lakes, such as Lake Constance, both abundance and leucine incorporation rates are significantly lower [0.004 to 0.035 \times 10^{10} \text{ (g DW)}^{-1} and 1 to 7 pmol (g DW h\(^{-1}\)) respectively \cite{Sala and Güde, 2006}. Therefore we conclude that, due to irregular disturbance, the water rich and often recently resettled particles on sediment surface have average or low (for highly eutrophic lake) abundance of highly active bacteria.

**Bacterial diversity in sediment**

Figure S1 compares the PCR-DGGE profiles of the two upper sediment layers. A total of 77 distinct bands [operational taxonomic units (OTUs)] were clearly detected within the whole set of samples. In single samples, 53–67 OTUs were observed. The highest number of OTUs (67) occurred on 12 June 2007 (0.5–1 cm) and the lowest (53) on 15 May 2007 (0.5–1 cm). Thirty-one ubiquitous OTUs were observed in all samples.

From the DGGE polyacrylamide gel, 18 ubiquitous OTUs were excised, resulting in 26 unique 16S rRNA gene fragments by DNA sequencing (similarity < 97%; Table S1A). OTUs with the same migration position were labelled with double identity; bands with a slightly different migration, but without visibly matching bands on the original DGGE gel, were omitted from the statistical analysis. Phylogenetic identification of sequenced DGGE bands was performed using the Ribosomal Database II (RDP II version 10, update 17) SeqMatch, or alternatively Green-Genes NAST alignment \cite{DeSantis et al. 2006}, and ARB parsimony \cite{Ludwig et al. 2004}. Unique sequences with < 97% similarity between each other (no bands had similarity between 97% and 99%) were interpreted as identified taxonomic units (ITUs). Both phylogenies matched well (Table S1), the most abundant being Proteobacteria and among them, Betaproteobacteria (11 ITUs). Three ITUs were identified as Gammaproteobacteria and three as Deltaproteobacteria. Other phylogenetic classes represented by a few ITUs were Bacteriodetes (2), Chloroflexi (2), Chlorobi (1) and WS3 (1). One DGGE band was identified as diatom chloroplast and two as Cyanobacteria. Similarity matches from RDP II originated from river, pond, mangrove and lake sediments (14 ITUs), or sediments from environments such as rice field soil, tundra and prairie soil, and sludge from waste water treatment plants or reactors. Only unclassified Anaerolineae Vol-S31-18 had closely related matches originating from marine sediments. All ITUs belonged to clades represented by uncultured organisms at the 97% similarity level.

In four cases, the migration of two phylogenetically loosely related DGGE bands was the same (on Fig. 2, S10-1/S29-16; S27-1/S27-9; S31-12/S31-26, S32-1/S32-4). dsDNA melting temperatures (Tm) calculated using a theoretical model (Poland algorithm, Steger, 1994) were similar (78.7°C and 78.6°C) for unclassified Chromatiales Vol-S10-1 and unclassified Betaproteobacterium Vol-S26-16, respectively, which migrated to the same position; the pairwise sequence similarity was 0.877. Unclassified Betaproteobacterium VoL-S32-1 and unclas-
sified Betaproteobacterium S32-4 bands migrated to the same position. The calculated Tm had also a 0.1°C difference (80.1°C and 80.0°C, respectively), with a pairwise sequence similarity of 0.920. Unclassified Anaerolineae VoL-S31-18 and unclassified Burkholderiales VoL-S31-27 migrated same, calculated Tm had a 0.3°C difference (79.0°C and 78.7°C, respectively), pairwise sequence similarity being 0.758. In two cases, band migration to the same position in DGGE gel did not correspond to the calculated Tm: Cyanobacterium VoL-S27-1 and unclassified Deltaproteobacterium VoL-S27-9 yielded a 0.8°C Tm difference (77.7°C and 76.9°C, respectively), with a pairwise sequence similarity of 0.920. Unclassified Betaproteobacterium S32-4 bands migrated to the same position; however, the calculated Tm had also a 0.1°C difference (79.2°C and 78.1°C, respectively), and Syntrophus sp. VoL-S31-26 and Xanthomonas sp. VoL-S31-27 yielded migrated to the same position; however, the calculated Tm were 79.2°C and 78.1°C.

After reamplification, four DGGE bands (Table S1B, S9-8; S31-18/S31-27; S41-4) migrated very close to the original position of the band to be excise, but nevertheless were obviously displaced. No other detectable bands could be identified at these positions on the original gel. Chromatograms of these sequence results were carefully checked and chimera analysis was performed using Bellerophon (Huber et al., 2004) at GreenGenes (DeSantis et al., 2006).

Determined phylogeny of ITUs was similar to shallow aquatic ecosystems (both sediment and water column) environments in the northern boreal zone. Similarly to previous studies, the sequences affiliated to Betaproteobacteria were most frequent (e.g. Stein et al., 2001; Briée et al., 2007). ITUs from L. Võrtsjärv were different from profundal sediments of deep lakes (e.g. Lake Constance; Pester et al., 2004), or from lakes at lower latitudes (e.g. L. Kinneret; Schwarz et al., 2007) and L. Taihu (Tang et al., 2009; Ye et al., 2009). Usually, sequences of Delta-Proteobacteria are frequently retrieved from sediments, but only a few sequences (3) were determined that affiliate to Delta-Proteobacteria in L. Võrtsjärv sediment. This could partly be explained by no attempt being made to analyse sequence information of all DGGE bands. On the other hand, most of Delta-Proteobacteria in sediments are sulfate reducers (e.g. Syntrophus, Desulfovibrio); they are abundant and play a cardinal role in anoxic settings, as in meromictic and anoxic lakes (Sass et al., 2002; Karr et al., 2005; Schwarz et al., 2007). Lack of abundant representatives in Delta-Proteobacteria may be due to an oxygen gradient between the usually aerated water body and the anoxic sediment below 1 cm. Some of bacteria might not tolerate frequent oxygenation of the upper sediment layer, while several sulfate-reducing bacteria are not strict anaerobes and prefer to respire oxygen when it is available (Sass et al., 2002). Phylotype belonging to the purple sulfur bacteria of the family Chromatiaceae (Gamma-proteobacteria) was detected as band VoL-S10-1 (Table S1). However, our phylotypes did not belong to the genus Achromatium, reported as common in German and English lakes (Gray et al., 1999). Phylotypes belonging to the Chloroflexi, typically associated with some anoxic settings, were found in L. Võrtsjärv sediment. The Chloroflexi are anoxygenic phototrophic filamentous bacteria that can be thermophilic and are frequently encountered in sulphide-rich environments (Elshahed et al., 2003), but environmental surveys have revealed many diverse phylotypes of these green non-sulfur bacteria in anoxic zones of stratified freshwater systems (Gich et al., 2002).

The diversity of bacteria belonging to highly divergent phylogenetic groups that contain no or very few cultured type species or even environmental isolates is quite high in freshwater sediments. We determined only one phylotype belonging to such divergent and uncertain lineages without cultivated members (WS3). This observation suggests that these phylogenetic groups are not among most dominant members of the upper sediment layers in L. Võrtsjärv. Particularly, no Gram-positive Actinobacteria or Firmicutes were found among phylotypes sequenced. Due to limited information about phylogenetic identity of bacterial species, detailed analysis of the relationship between environmental parameters and phylotypes is not possible. Interestingly, both Bacteroidetes ITUs identified by 16S rDNA sequencing (VoL-S39-7 and S40-5) were associated with higher DO in winter is in parallel to colder temperatures (Fig. 3B).
Dependence of bacterial community composition (BCC) on environmental factors

Correspondence analysis on a binary matrix (0 – band is missing from this position; 1 – band exists) of OTUs and ITUs per lanes was performed; groupings of the samples is given on Fig. 2. Community composition of OTU/ITUs showed not statistically difference between the two layers of sediment (upper 0.5 cm vs. lower 0.5–1 cm layer, multivariate between-groups analysis, Monte-Carlo permutation test $n = 1000$, $P > 0.05$). Community composition between ice-free and ice-covered periods was significantly different (multivariate between-groups analysis, Monte-Carlo permutation test $n = 1000$, $P < 0.001$).

To determine the association of the environmental variables with changes in bacterial community structure, the CCA was applied. Two first CCA axes explained 32.1% and 20.2% of the total variation in bacterial community structure, a relationship that was statistically significant (Monte-Carlo permutation test $n = 1000$, $P < 0.05$). The major part of the variance in the first CCA (CCA 1) axis was explained by temperature and DO concentration in the sediment. In addition, the first axis of CCA was negatively correlated with temperature ($r = -0.90$, $P < 0.001$) and moderately with Chl a concentration in the water column ($r = -0.55$, $P = 0.05$), while the axis 2 (CCA 2) was positively correlated with BP ($r = 0.69$, $P < 0.01$).

Seven ITUs correlated significantly with measured environmental variables (Fig. 3B); most of them were related to CCA 1. Bacteriodetes ITUs (VoL-S39-7 and VoL-S40-5) were favoured by the high DO concentration in winter. Group WS3 bacterium VoL-S51-17 and Betaproteobacterium VoL-S15-6 were related to late summer conditions with relatively high temperature and low oxygen concentration. Chloroflexi VoL-S14-29 and Gamma-proteobacterium VoL-S3-6 were preferably observed in samples from April and May when bacterial productivity started to increase and the number of ITUs/OTUs was lower.

Similarly to marine benthic habitats (see studies by Franco et al., 2007; Hewson et al., 2007; Böer et al., 2009), the BCC and activity were expected to be driven by various abiotic and biotic conditions in the sediment and lake water. Morphological diversity of bacteria tends to be higher in conditions with intermediate disturbance (Kassen et al., 2000). However, there is no evidence that this applies to phylogenetic richness. Undisturbed sediment has a strong vertical physico-chemical gradient that follows the redox potentials of the available electron acceptors. In the case of the oxic sediment-water interface, the steep gradient of DO concentration is observed in the upper layers of sediment. This leads to different mineralization processes driven by microbes, and therefore the bacterial community was expected to change within such a gradient.

Fig. 3. Canonical correspondence analysis (CCA) between binary matrix of presence/absence of DGGE bands and other descriptive parameters measured in sediment (BP – bacterial production and TNB – total number of bacteria in layer 0–0.5 cm; O$_2$ sed – dissolved oxygen penetration depth in sediment, Temp – temperature at the surface of sediment) or in water column (Chl a – chlorophyll a concentration; O$_2$ bot – dissolved oxygen concentration in water – 10 cm above sediment). Statistical relationships of CCA were significant ($P = 0.03$). Arrows represent correlations of descriptive parameters with CCA axes.

A. Sample scores labelled with dates. The time series is marked by the dotted line.

B. Species scores of ITUs. Species scores that had no relationship to explanatory variables are labelled by ‘several ITUs’.
During the ice-covered period, the response of the bacterial community to the relatively stable conditions corresponding to the formation of the redox gradient was expected in L. Võrtsjärv. Our study showed no switch in community composition between the upper and lower 5 mm thick sediment layer (Figs S1 and 2). In large shallow lakes, wind-driven resuspension is expected to have a strong influence on the upper layer of sediment (Bloesch, 1995). Because resuspension events occur irregularly over short timescale (hours to days), sampling frequency (monthly measurements) does not allow one to estimate the direct effect of wind events. However, we can compare two contrasting periods: ice-covered (no waves) and ice-free (with resuspension events being irregularly distributed over the period). Indeed, similar to sandy tidal flats (Böer et al., 2009), where environmental variables could not explain the distribution of bacterial species, frequent and irregular resuspension events shadowed the impact of other environmental factors on BCC. Unlike the above mentioned sandy tidal flat, the strong physical disturbance modulated the homogeneity in BCC over 1 year period. In correspondence analysis, two clusters were formed following roughly the ice-free versus ice-covered period with a 1 month time lag (Fig. 2). In 2007, ice-break occurred in March 29. Sediment bacterial community under the ice had low and stable activity, and a decrease in abundance together with the disappearance of several phylotypes (the total number of phylotypes dropped by seven OTUs) was observed. Monotonous decrease of DO concentration towards the end of winter was the obvious most regular year-by-year recurring change in abiotic conditions. In L. Võrtsjärv, DO depletion often also occurs in bottom layers of water column by the end of ice-covered period (Tuvikene et al., 2002), assuring anoxic sediment. Interestingly, only a small proportion of bacterial community responded to such changes in the environmental conditions, suggesting that most part of the community is adapted for highly disturbed conditions and has a high tolerance for the presence or absence of DO. This observation suggests that only limited number of species do not tolerate conditions occurring at the end of ice cover. Therefore, it is not surprising that the seasonal dynamics of BCC was positively correlated with DO in sediment (O₂ sed) and negatively with temperature (Fig. 3A). In addition, seasonal dynamics of BCC followed the changes in activity of bacteria (BP) from spring to summer. At the end of summer (August), changes in BCC were significantly associated with a greater abundance of bacteria and an increase in phytoplankton abundance (Chla concentration).

Conclusions and outlook

The community composition of bacteria in the upper 1 cm of the sediment proved to be rich in high number of phylotypes, but most of phylotypes were common in all samples over 1 year period. Beta-proteobacteria, known as common fresh water column bacteria, were predominant among the identified phylotypes in sediment, although other proteobacteria were also identified. Identification of bacteria based on ribosomal RNA gene sequences did not allow the analysis about specific functional groups and their activity. However, the relatively homogeneous community of bacteria responded with dynamic abundance and activity to pronounced seasonality of physical environment (temperature and DO concentration), and to the abundance of phytoplankton (Chl a concentration). Absence of wind-driven resuspension under permanent ice and concurrent depletion of DO in sediment caused a statistically significant, but minor, decrease in richness of the bacterial community. These observations are interpreted as indications about strong interaction between upper layer of sediment and water column; these interactions are modulated by resuspension events. Under ice conditions the community did not change irreversibly therefore speculate that differently from deep lakes new settled material is not buried into sediment instead remains in close contact and therefore shares the bacterial species with water column. This has to be tested by comparative study on resuspended particles in water column.

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**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** PCR-DGGE profiles in the upper sediment layers (0–0.5 cm and 0.5–1 cm) over the period from October 2006 to October 2007. ITU codes are the same as in Table S1.

**Table S1.** Closest relatives (accession number of closest relative in GenBank) of sequenced 22 DGGE bands, which
had a detectable mate on the original DGGE gel. The RDP II SeqMatch tool was used to identify the closest match among cultures and isolates with sequences of quality ‘good’ and length > 1200 bp. For matching sequences, the GenBank accession number (Acc nr), similarity score (S_score), phylogenetic lineage (Phyla and Class) and phylogenetic identity (Phylo_ID) are given. ARB phylogenetic lineage (ARB lineage) shows phylogenetic affiliation in the global ARB tree (additional lineage at second row for each ITU);

B. Four phyloptypes that lacked detectable mates on the DGGE gel.

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